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MODULATION OF THE NUTRITIONAL CONTEXT AND EARLY EXPERIENCE  
AS NEW TOOLS TO INCREASE THE USE OF MEDUSAHEAD (*TAENIATHERUM*  
*CAPUT-MEDUSAE* SSP. *ASPERUM*) BY GRAZING SHEEP

by

Juan J. Montes

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Range Science

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Logan, Utah

2016

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## ABSTRACT

Modulation of the Nutritional Context and Early Experience as New Tools to Increase the  
Use of Medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) by Grazing Sheep

by

Juan J. Montes, Doctor of Philosophy

Utah State University, 2016

Major Professor: Dr. Juan J. Villalba  
Department: Wildland Resources

The success of medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) as an invasive exotic grass in the western US is attributed in part to its low palatability. The nutritional context where medusahead grows can be modulated by the use of supplements that increase herbivores' preference for unpalatable feeds. Additionally, positive experiences early in life (with mother, with supplements) can have long-life influences on preference for unpalatable feeds. To test the influence of the nutritional context on medusahead intake, ewes grazed with their lambs during summer of 2013 on medusahead-infested rangeland with (Treatment) or without (Control) the daily provision of an energy-rich supplement. To test for the effect of experience early in life at grazing medusahead on use of this weed later in life, lambs that grazed with their mothers during 2013 (Experienced) were exposed to medusahead (in pens and during grazing) as yearlings during summer of 2014 along with inexperienced (Control) animals. To better understand the unpalatability of medusahead, the fermentation kinetics of medusahead at

different phenological stages and particle sizes was assessed. Ewes grazing with their lambs showed low use of medusahead (5% of the grazing events recorded), even when supplemented. Nevertheless, medusahead use increased across the grazing period and utilization was similar to medusahead abundance in the plant community. Use of medusahead by nursing lambs was correlated with that observed by their mothers and lambs utilized medusahead to the same extent either before or after weaning. Yearlings in pens showed low intake of medusahead and a cyclic pattern of intake across days. However, experienced yearlings displayed a more even intake of medusahead across days and a greater gain-to-feed ratio than Control yearlings. All yearlings showed low to nil use of medusahead during grazing. Medusahead had lower fermentation rates than alfalfa hay and fermentation rates declined with plant maturity. Organic matter digestibility for medusahead declined as particle size of the substrate increased, a relationship that explains the low palatability of the weed. These results provide the foundation for grazing treatments aimed at reducing the abundance of the weed or at preventing its spread in rangelands with different levels of medusahead infestations.

## PUBLIC ABSTRACT

Modulation of the Nutritional Context and Early Experience as New Tools to Increase the  
Use of Medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) by Grazing Sheep

Juan J. Montes

Medusahead is an invasive weed that reduces wildlife habitat and biodiversity as well as commercial and recreational value of land. Grazing represents a sustainable method for its control but stakeholders claim that livestock will not eat medusahead because of its low feed value. This research explored a supplementation program, along with experiences early in life with mother to enhance use of medusahead by sheep. Results showed that an energy supplement did not enhance medusahead use by sheep but that early experience with mother influenced yearlings to use the weed more evenly across days. When availability of the weed was low at pasture sheep did not reject medusahead and consumed the weed in proportion to its abundance. Thus, prudent continuous grazing treatments may be used to reduce the spread of medusahead when the levels of infestation are low (e.g., < 10% abundance). When infestations are high (e.g., > 70% abundance), managers should create grazing cycles where experienced sheep graze medusahead patches for a short time (e.g., 1 day), then a diverse vegetation for 3-7 days, and then medusahead for another short time, mimicking the cyclic pattern of medusahead intake. Finally, ewes that showed a high medusahead use (up to 12%) could be selected to create homogeneous flocks with greater preference for the weed.

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Juan J. Montes

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## CHAPTER 1

### INTRODUCTION

#### **Plant Invasion**

Invasion of natural landscapes by exotic plant species is a major threat to ecosystem functioning and as a consequence, a significant problem for land managers (Young *et al.*, 1999; Masters and Sheley, 2001). Disturbance of native plant communities, whether natural or induced by management, is a primary factor contributing to successful invasion by exotic plant species (Masters and Sheley, 2001; Norton *et al.*, 2007). For instance, wild and domestic ungulate herbivores may exert considerable disturbance on the structure and composition of native plant communities (Hobbs, 1996), which can potentially promote the establishment and spread of exotic plant species. Specifically, herbivores may favor weed invasion by: (1) transport of seeds via endozoochory and epizoochory; (2) disturbances caused by feeding and trampling, (3) feed selection, and (4) intensity, time and timing of herbivory. Regarding feed selection by ungulates, exotic plants are typically unpalatable and as a consequence their competitive ability is favored as palatable native plants are selectively ingested by grazers (O'Reagain and Grau, 1995; Vavra *et al.*, 2007). Additionally, intensity, time and timing of grazing can greatly affect plant productivity and vigor (O'Reagain and Grau, 1995; Vavra *et al.*, 2007).

The different native ecosystems of the United States have been invaded by a diversity of annual plant species with negative impacts on their ecological services. For instance, *Taeniatherum caput-medusae* ssp. *asperum* is an annual grass that modifies the assemblages of native vegetation, leading to substantial changes in the physical and biological potential of rangelands in the northwest of the United States (Bovey *et al.*,

1961; Young, 1992). The low intake and palatability of *T. caput-medusae* by grazers increases the grazing pressure on native plant species, which reduces carrying capacity (Hironaka, 1961; Torrel *et al.*, 1961). Furthermore, the dead matter of the weed accumulates as slowly-degradable litter (i.e., thatch), which increases fire frequency in the invaded landscapes favoring its spread across annual and perennial native plant communities (Hironaka and Sindelar, 1973; Young, 1992).

### ***Taeniatherum caput-medusae* ssp. *asperum* – Medusahead Grass**

#### **Distribution**

*Taeniatherum* Nevski is a well-defined annual, self-fertile, diploid grass genus of the tribe Triticeae. In its natural habitat, *Taeniatherum* extends across the Mediterranean region, from Portugal to Central Asia and North of Africa (Frederiksen and von Bothmer, 1986). *Taeniatherum caput-medusae* is the only species in this genus, which has three subspecies: *caput-medusae*, *crinitum*, and *asperum* (Frederiksen and Von Bothmer, 1986; Savard *et al.*, 2003). The subspecies *caput-medusae* is mostly restricted to Portugal, Spain, southern France, Morocco, and Algeria (Ruíz-Fernández and Soler, 1997); subspecies *crinitum* is from Greece and Yugoslavia eastward into Asia, and *asperum* completely overlaps the distribution of the other two subspecies (Frederiksen and Von Bothmer, 1986). Native populations of *T. caput-medusae* have a high degree of local genetic differentiation possibly by adaptation to local conditions, resulting in low-fertile plants by crossing subspecies and into *asperum* subspecies (Frederiksen and Von Bothmer, 1986).

*Taeniatherum caput-medusae* ssp. *asperum* has been invading slowly and continuously thousands of acres in the western United States since its first report in 1887 (Young, 1992). From that time until 2005, it has been estimated that medusahead infested over 2.35 million of acres in the western states (Duncan and Clark, 2005), such as Oregon, California, Washington, Idaho, Nevada, and Utah (Young, 1992; Davies and Johnson, 2008). This weed continues to expand its range by about 12% per year (Duncan and Clark, 2005) and over 62 million acres are at risk of invasion (Johnson and Davies, 2012). Novak (2004) suggested that Usanian medusahead populations have a European origin; the common name of *T. caput-medusae* spp. *asperum* is medusahead.

#### Environmental Characteristics of Medusahead in the Western United States

Medusahead is well adapted to clay, stony and well-developed soils (Dahl and Tisdale, 1975), which hold water in hot springs for medusahead's deep root system (Harris, 1977), contrasting with the shallow roots of seedlings in native grasses and forbs. In addition, medusahead is known for its great tolerance to extreme temperatures (Mangla *et al.*, 2011) and for its inherent capacity to extract moisture from extremely dry soils (Young *et al.*, 1999). This is likely the reason medusahead grows 2-3 weeks later than other native species and exotic annual weeds (i.e., *Bromus tectorum*), which in turn creates a gap between the time for greater water and nutrient requirements of many native plants and medusahead.

Seasonal precipitation is of greater significance than total precipitation regarding water requirements of different plant species (Torrel *et al.*, 1961), spring rains favor native grasses, whereas winter rains favor medusahead growth (Young *et al.*, 1999; Sheley and James, 2010). In addition, some clay soils expand and shrink with the

presence and absence of water, causing native plant seeds to fall into deeper soil, and stony soils create an unfavorable environment for germination of bunch grasses (Young *et al.*, 1999).

Medusahead thatch works in different ways to favor invasion. It creates a better seedbed (e.g., a micro-environment with greater humidity and temperature) for medusahead seeds (Hironaka and Sindelar, 1973; Young, 1992) than bare clay soil. The thick thatch retains more water for germination of medusahead seeds and radicles can later penetrate through the thatch into the soil surface, a process that does not occur in other plant species (Hironaka, 1961; Young *et al.*, 1999). In addition, medusahead thatch is a source of fuel for fire and medusahead seeds are resistant to fire, while native plants in general are not (Young, 1992; Young *et al.*, 1999).

#### Medusahead Intake by Ungulate Herbivores

The intake of medusahead by domestic herbivores is variable, but generally low. *T. caput-medusae* is used as forage on its natural habitat in Europe and Asia (Shawrang and Nikkhah, 2005; Yiakoulaki *et al.*, 2009). Yiakoulaki *et al.* (2009) found in silvopastoral systems of Greece that this grass represented 6.4% of the diet in sheep and 4.6% of the diet in goats and this grass was among the four grasses most consumed out of eleven available in the community.

Medusahead intake is typically low by domestic sheep in the relatively new habitat of the western US. Lusk *et al.* (1961) reported that sheep consumed medusahead during the vegetative stage, although palatability decreased with plant maturity, as they ate 52% of the selected diet as medusahead at the vegetative stage and 24% at the headed stage. The same authors found that fertilized medusahead plants were grazed to a greater



extent than unfertilized medusahead plants. Young (1992) reported that livestock consumed very little amounts of medusahead at any stage of its growing cycle, reducing carrying capacity by up to 80% while favoring its persistence through its litter.

### Chemical Composition

Medusahead has a chemical composition (on a dry matter [DM] basis) similar to other grass species. Bovey *et al.* (1961) reported concentrations (ranging from the vegetative to the flowering stage, respectively) of 10.4 to 6.8% crude protein (CP), 2.6 to 1.8% fat, 26.8 to 27.4% crude fiber, 6.1 to 8.6% lignin, and 12.8 to 13.9% ash. Van Dyne (1962) found that medusahead had 27% of cellulose in the boot stage and 32% in the mature stage. Shawrang and Nikkhah (2005) recorded 12.4% CP and 51.2% neutral detergent fiber (NDF) in the plant, but they did not describe the phenological stage. Other results showed 10.8 to 8.5% CP, 62.0 to 58.2% NDF, and 46.3 to 37.8% acid detergent fiber (ADF) from late vegetative stage to late reproductive stage, respectively (Villalba and Burritt, 2015).

Medusahead has a high concentration of silica in its tissues, ranging between 11.8 to 18.5% DM, representing from 70 to 93% of medusahead's mineral fraction (e.g., ash) (Bovey *et al.*, 1961; Swenson *et al.*, 1964). Villalba and Burritt (2015) reported concentrations of 8.1 to 16.0% of acid insoluble ash (AIA) during the late vegetative and reproductive stage of the plant, respectively. Acid insoluble ash is a fraction of the plant's tissues which comprises the inorganic material not solubilized in acid, with silica as its major component (> 90%) (Arcos *et al.*, 2007; Charca *et al.*, 2007).

## Silica in Plants

### Absorption and Distribution in Plant Tissues

Silica is absorbed as orthosilicic acid ( $\text{Si}[\text{OH}]_4$  – soluble silica) by plant roots and transported throughout plant tissues with the transpiration stream (passive uptake) (Neethirajan *et al.*, 2009). Since silica accumulation is directly correlated with transpiration, juvenile leaves with a high photosynthetic capacity and greater transpiration rates increase silica deposition rates (Raven, 2003). Shewmaker *et al.* (1989) reported for semiarid grasses greater silica concentrations in leaves, intermediate in inflorescences, and lower in stems, being the same order for water loss by transpiration. Plant silica content varies as a function of the plant's tissues, but also as a function of species, phenological stage, defoliation pressure (McNaughton *et al.*, 1985; Shewmaker *et al.*, 1989), and availability of silica in the seedbed (Massey *et al.*, 2007). There is also some evidence suggesting that energy mediates the uptake of silica in grasses (Ma *et al.*, 2004).

Silica can be found in plants as: a) undeposited, soluble, free orthosilicic acid, and b) solid, amorphous and relatively insoluble bodies of polymerized orthosilicic acid (opal phytoliths,  $[\text{SiO}_x(\text{OH})_{4-2x}]_n$ ) in the lumen and cell walls, and intercellular spaces or external layers (Jones, 1978; Bailey, 1981).

Swenson *et al.* (1964) analyzed the silica content in medusahead and found: 1) the form of silica was opal; 2) silica was evident in the cell walls of the epidermis of the leaves, awns, glumes, and seeds and particularly on the barbs of the awns, and 3) strands of sausage-shaped opal deposits comprising the inner structure beneath the epidermis in

the awns and stems parallel to the surface. However, these authors did not make any comment about the presence of soluble silica in medusahead's tissues.

### Silica Content

According to silica content, plant species are classified as either silica accumulators or non-accumulators (Neethinrajan *et al.*, 2009). Dicotyledonous plants present low silica contents (Vicari and Bazely, 1993). Johnston *et al.* (1968) reported values lower than 1.5% DM in shrubs and forbs. On the other hand, grasses accumulate a wide range of silica in their tissues. For instance, Olubajo *et al.* (1974) and Johnston *et al.* (1968) reported for African tropical grasses and European perennial grasses values of silica concentration no greater than 4% DM. Shewmaker *et al.* (1989) reported intermediate concentrations in rangeland grasses (3.5-7.3% DM). Nevertheless, grasses may also show a high concentration of silica in their tissues; McNaughton *et al.* (1985) reported values of silica concentration ranging from 11.9 to 19.6% DM in African savanna grasses, similar to concentrations reported for medusahead, 11.8 to 18.5% (Bovey *et al.*, 1961; Mutch and Philpot, 1970). Swenson *et al.* (1964) concluded that medusahead is one of the most effective silica accumulators ever reported.

### Silica Functions

Silica has different functions in plants, such as conferring tissue stiffness to support the shoot and providing mechanical strength and rigidity of the leaves to reduce trampling and susceptibility to herbivory (McNaughton *et al.*, 1985; Vicari and Bazely, 1993).

Silica deposits function as a physical barrier to enzymatic degradation by fungal pathogens (Currie and Perry, 2007). Silicon is also a biologically active element capable

of triggering a broad range of natural defenses (innate immune system), enhancing the activity of chitinases, peroxidases, polyphenol oxidases, and flavonoid phytoalexins, and the production of glycosylated phenolics and diterpene phytoalexins, all chemicals with functions to protect plant tissues against fungal pathogens (Chérif *et al.*, 1994; Currie and Perry, 2007).

Toxicity by high concentrations of Mn, Cd, Al, and Zn in plants can be alleviated by silica applications to the seedbed (Epstein, 1999; Ma, 2004). Martínez-Ruiz *et al.* (2001) and Reglero *et al.* (2008) reported medusahead as a colonizer in European mine wastelands, soils with high concentration of heavy metals.

Silica bodies conserve water during moisture stress or drought, and they have been shown to influence stomata movement at the plant leaf epidermis with reductions in the transpiration rate (Neethirajan *et al.*, 2009). Increased drought tolerance by the application of silica to soils may result from decreased transpiration and from the presence of silicified structures in plants, which provide an effective cooling mechanism, thereby improving plant tolerance to high temperatures. These functions indicate the importance of silica accumulation in some plants and the ability of medusahead to grow 2-3 weeks later than other plants species (Young, 1992), when availability of soil water declines and ambient temperatures increase.

Silica promotes cell elongation in the growing zone and decreases cell-wall extensibility in the basal zone of stellar tissues in the roots, which enhance root elongation in plants (Neethirajan *et al.*, 2009). These characteristics likely explain the deep root growth of medusahead seedlings in the fall, as reported by Hironaka (1961) and Harris (1977).

McNaughton *et al.* (1985) suggested silica is a true growth promoter for some perennial grasses, possibly contributing to a more favorable carbon balance in plants by substituting carbon-generated support for mineral-generated support. In support of this statement, Van Soest (2006) cited that accumulation of structural carbohydrates is ten-fold more (metabolically) expensive than silica accumulation in rice plants, and Van Soest and Jones (1968) suggested that plants tended to produce less lignin on soils of high silica availability. In addition to contributing to the plant's metabolic economy, silica may reduce the amount of tissue lost to herbivores during grazing, as it reduces forage intake by herbivores (Maylad and Shewmaker, 2001; Massey *et al.*, 2009) by decreasing forage digestibility (Van Soest and Jones, 1968; Smith *et al.*, 1971).

#### Silica and Forage Digestibility

Silica reduces digestibility of and preference for grasses by chewing invertebrates (Massey *et al.*, 2006; Hunt *et al.*, 2008), voles (Massey and Hartley, 2006; Massey *et al.*, 2008), rabbits (Cotterill *et al.*, 2007), and sheep (Massey *et al.*, 2009). These animals showed relatively low growth rate and survival, losses in body mass, and reduction in reproduction parameters and population size when ingesting grasses with concentrations of approximately 8.0% silica (DM basis). Van Soest and Jones (1968) and Smith *et al.* (1971) estimated that per unit of increase in silica content of a grass, there is between one to three units of decline in *in vitro* DM digestibility.

Amorphous silica depresses the digestibility of grasses by acting as a varnish on the plant cell wall, reducing accessibility to rumen microorganisms, which ferment structural carbohydrates (Van Soest and Jones, 1968; Mayland and Shewmaker, 2001). Furthermore, soluble silica reduces activity of digestive enzymes (Kind *et al.*, 1954), such

as cellulase (Shimojo and Goto, 1989), which in turn inhibits forage digestibility (Smith and Nelson, 1975), likely by adsorbing cations, basic amino acids, peptides, and proteins (Patwardhan *et al.*, 2012; Prabowo and Spears, 1992).

The aforementioned characteristics suggest that the low intake of medusahead by herbivores is caused by the high silica content in its tissues, which negatively impact digestibility. Nevertheless, Shawrang and Nikkhah (2005) recorded DM and organic matter (OM) digestibilities in *T. caput-medusae* (unknown ssp.) greater than 65%, although these authors did not report either the phenological stage of the grass or its silica content. Van Dyne (1962) found that immature medusahead had greater *in vitro* cellulose digestibility (79-82%) than mature medusahead (70-72%), mature mixed annual rangeland (67%), and alfalfa (51%), again, without reporting values for silica content. It is worth noting that this experiment used purified cellulose, likely eliminating the structural (e.g., a barrier that reduces bacterial access to cell walls) effect of amorphous silica in inhibiting digestibility.

Shewmaker *et al.* (1989) found that preference for different semiarid grasses by sheep was not related to soluble, insoluble, or total silicon concentration at any phenological stage of the forages assayed; suggesting that preference for these grasses was a function of factors other than silicon, such as lignin. McNaughton *et al.* (1985) found that wildebeest preferred high-silica bunch grasses (>10%) over grasses with low silica content, while Brizuela *et al.* (1986) reported a similar pattern for bison, although silica content was lower for the grasses in that study (<4%). Both research teams suggested that preference for high-silica grasses was due to the long grazing history of herbivores in such ecosystems.

## **Medusahead Control**

Control and/or eradication of medusahead are main goals for rangelands in the western US. Different control methodologies have been evaluated, including fire, chemical, mechanical, biological, and cultural approaches. All of these control methods entail different costs and degrees of success, which depend on environmental conditions (e.g., rain distribution), degree of infestation, abundance of native plants, and period of application (Sheley and James, 2010; James *et al.*, 2015).

An important point to consider for the control of medusahead in rangelands is the reduction in the dispersion of the weed to new locations across the landscape (Davies and Johnson, 2008). It has been recommended to establish a plant barrier to avoid dispersion and increase competition; Davies (2008) suggested the establishment of a barrier around medusahead patches with tall bunch grasses such as bluebunch wheatgrass (*Elymus spicatus*). This grass, as well as other native tall bunch grasses, has the capacity to compete with medusahead (Harris, 1977; Sheley and James, 2010); however, there is barely to find recruitments on rangeland (Young *et al.*, 1999). Bunch grass seeds germinate in the fall and seedlings grow in the spring favored by spring rains (Harris, 1977). Sagebrush can also be transplanted to increase populations and start changes in the microenvironment of the sagebrush understory (Young *et al.*, 1999). Preventing grazing from July to October as well as avoiding the presence of fomites (i.e., vehicles) can decrease the spread of medusahead when seeds disarticulate from the inflorescence (Davies, 2008).

Prescribed burns in the fall with a later spray of pre-emergent herbicide (Imazapic) is a good combination for medusahead control (Davies and Sheley, 2011),

although an undesirable effect of imazapic is the reduction of annual forb abundance (Davies and Sheley, 2011). The success of this treatment depends on the residual native vegetation; if there is enough native vegetation, then tall perennial bunch grasses increase in abundance, which has been demonstrated to be the most important native plant functional group to prevent exotic annual grass invasion (Davies and Sheley, 2011). Spring prescribed grazing preceding fall burning might decrease the biomass of medusahead and decrease the negative effects of fire on desirable plants.

Numerous studies have tested the utility of prescribed livestock grazing, particularly with sheep and goats to reduce biomass of invasive plants. Cattle appear less effective at reducing invasive plants than sheep or goats (Frost and Launchbaugh, 2003; Vavra *et al.*, 2007). DiTomaso *et al.* (2008) found that high sheep densities (6.7 animals/month/ha) were very effective at reducing medusahead at the stem elongation stage on heavily infested rangeland (50-70% medusahead cover). Nevertheless, high animal densities are unrealistic for managing large medusahead infestations. Furthermore, considering the low intake of medusahead observed by forcing lambs to eat this weed in individual pens (Hamilton *et al.*, 2015; Villalba and Burritt, 2015) or during grazing (Lusk *et al.*, 1961), it is likely that the effect of high-stocking densities on reductions of medusahead abundance are more a consequence of trampling than grazing. Even if animals ingest more medusahead at high stocking densities, it is important to know how long they will consume the weed without experiencing the adverse effects of plant silica on digestibility, and ultimately the negative impacts of the weed on animal welfare and production, as discussed in previous sections (Massey and Hartley, 2006; Massey *et al.*, 2009).



### **Food Selection and Nutritional Context**

From a traditional foraging perspective, herbivores grazing in diverse plant communities select those species with greater nutritional quality than the average available in the environment (O'Reagain and Grau, 1995; Vavra *et al.*, 2007). Less preferred species are consumed after the more preferred species in the sward have been depleted (O'Reagain and Grau, 1995). This traditional view treats each forage species as an isolated entity without taking into consideration new paradigms in foraging behavior such as the importance of positive experiences early in life with the biochemical context (provided by the plant community or supplements) on preference for target plants. Preference for a particular food depends not only on its intrinsic (e.g., nutritional, toxicological) properties, but also on the nutritional context where that food is ingested. Thus, preference for a target plant species is modulated by the chemical composition of the plants present in the associated community (Flaherty, 1996; Provenza *et al.*, 2003). The biochemical context in which unpalatable foods are consumed is critical for enhancing their use and preference by herbivores. The specific array of foods encountered and the sequence of encounters could turn out to be crucial in determining an animal's food preferences (Villalba *et al.*, 2006; Bergvall and Balogh, 2009). These contextual effects may occur due to the digestive interaction among feeds, intake induction, or the interplay between these mechanisms (Provenza *et al.*, 2003; Freidin *et al.*, 2011).

Intake induction or facilitation results when animals repeatedly ingest a less preferred food in association with a highly preferred food. It has been shown that sheep increase their intake of and preference for low-nutrient or phytochemical-containing

foods closely paired with meals of higher nutritional quality (Villalba *et al.*, 2006; Freidin *et al.*, 2011).

Supplementation modulates forage intake (Caton and Dhuyvetter, 1997; Garcés-Yépez *et al.*, 1997). Supplementing low-quality feeds to maximize intake and nutrient utilization is key to enhance animal performance. Supplements containing highly digestible fiber (i.e., soybean hulls, beet pulp, and wheat midds) fed at low levels increase intake of low-quality forages by livestock (Caton and Dhuyvetter, 1997). On the other hand, high-starch supplements like corn can cause negative associative effects that constrain food intake (Caton and Dhuyvetter, 1997; Garcés-Yépez *et al.*, 1997). Supplementation with a readily degradable fiber source has been suggested as an option for maintaining rumen stability in regard to fiber digestion, rumen pH, and for minimizing intake reductions associated with grain supplementation (Cooper *et al.*, 1996; Caton and Dhuyvetter, 1997).

High-energy and high-protein concentrates fed to ewes increased use of sagebrush (2X) relative to non-supplemented animals (Dziba *et al.*, 2007). Supplemented ewes spent more time feeding on sagebrush likely due to increased efficiency of detoxification of terpenes from adequate supply of nutrients required for conjugation and elimination processes (Dziba *et al.*, 2007). Supplements, therefore, offer the potential to increase intake of plants that are generally avoided (Provenza *et al.*, 2003; Dziba *et al.*, 2007).

In addition to the positive effects of the nutritional context on use of unpalatable feeds, positive experiences early in life (with mother, with the appropriate supplement) can have life-long influences on herbivores by causing neurological, morphological, and physiological changes that influence foraging behavior (Provenza and Balph, 1990). By

interacting with the genome during growth and development, social and biophysical environments influence gene expression and behavioral responses (LeDoux, 2002; Fish *et al.*, 2004). Thus, given the appropriate context early in life, animals may increase consumption of medusahead and its use may persist throughout the life of the individual and across generations.

Goats reared with their mothers on blackbrush-dominated land from 1 to 4 months of age consumed over 2.5 times more blackbrush than did goats naïve to blackbrush; experienced goats still consumed 30% more blackbrush than inexperienced goats when allowed to choose between poorly nutritious blackbrush and alfalfa pellets (Distel and Provenza, 1991). Distel *et al.* (1994) reported changes in digestibility caused by experiences early in life with low-quality feeds; the apparent digestibility of sorghum hay was 4.5% greater in experienced lambs (55.1%) than in inexperienced lambs (50.6%). Furthermore, the refused roughage in inexperienced lambs had 3.7% more NDF content than refused roughage from experienced lambs, suggesting that early experience with a low-quality feed enhanced the lambs' ability to consume more fiber with their diet.

Associative effects involving plant chemistry and herbivore learning influence the coexistence of plants and animals, and can either enhance or diminish biodiversity. It has been suggested that biochemical diversity increases the potential for resiliency, adaptability, and productivity of ecosystems by increasing options for plants, herbivores, and people (Provenza *et al.*, 2003). A greater array of options for herbivores involves a more balanced diet with nutrients and phytochemicals, which improve animal nutrition, health and welfare, and a more even utilization of plant resources (i.e., palatable and

unpalatable plants) that contributes to enhance and maintain the biodiversity of the landscape (Provenza *et al.*, 2003).

Medusahead is a clear problem in US rangelands, as it has been continuously spreading since the first reports of its presence dating from the 1800's, threatening habitat, biodiversity, and ecological services. Due to its invasiveness, producers refer to medusahead as a “devil” species with the potential to take over much of the native grassland in the west of US (DeLong, 2011). Chemical control of the weed can be effective, but it has potential negative side effects such as undesirable impacts on soils and native plants. Moreover, chemical control is often temporary in nature (Davies and Johnson, 2008) and the need for repeated applications makes it unaffordable for many ranchers.

## **Objectives**

Grazing represents a sustainable, efficient, and low-cost alternative for the control of medusahead in western rangelands. In addition to plant use, grazing influences the nitrogen (N) cycle by changing litter quality, thereby affecting conditions for N mineralization, and by adding readily available N to upper levels of the soil in urine and feces (Hobbs, 1996). In fact, stakeholders listed grazing as a preferred management tool to control medusahead in California (James et al., 2015). However, given the unpalatability of the weed and the aforementioned contradictory results about the effectiveness of grazing at controlling medusahead, there is a clear need to better understand the mechanisms and processes underlying medusahead intake by herbivores in order to devise new management plans that optimize grazing animal impacts on medusahead.

Here I propose to: Create the appropriate nutritional context through a diet supplement that enhances use of medusahead by grazing ewes and their lambs (Objective 1), test the effects of such nutritional context experienced early in life with mother (during Objective 1) on use of medusahead later in life by sheep (Objective 2), and finally measure the digestibility and digestibility kinetics of medusahead at different phenological stages and particle sizes in order to better understand utilization of this weed by grazing ruminants (Objective 3).

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CHAPTER 2

EFFECT OF AN ENERGY SUPPLEMENT ON USE OF MEDUSAHEAD  
(*TAENIATHERUM CAPUT-MEDUSAE* SUBSP. *ASPERUM*) BY  
GRAZING EWES AND THEIR LAMBS<sup>1</sup>

**Abstract**

This study explored the effect of energy supplementation on use of medusahead, an invasive annual weed, by ewes and their lambs. Thirty-six ewes with their lambs (2-3 months old) were randomly assigned to 12 groups (3 ewes with their lambs per group) and half of the groups received 2.5 kg group d<sup>-1</sup> of an energy-based supplement (beet pulp:barley: Ca-propionate, 66:30:4; as-fed basis). After supplementation, all groups grazed plots with medusahead infestation for 15 days. Lambs were then weaned, kept in the same groups but without supplementation and allowed to graze medusahead-infested plots for 3 days. Grazing events were recorded daily at 5-min intervals and defoliation of medusahead tillers was measured in all plots. The proportion of grazing events recorded on medusahead and the proportion of defoliated medusahead tillers were not affected by supplementation in either ewes or lambs ( $P > 0.05$ ). All ewe-lamb groups increased the proportion of grazing events on medusahead as the trial progressed, with a greater proportion of medusahead use during the second half of the grazing period ( $P < 0.05$ ). Nevertheless, the average proportion of events recorded for medusahead use was never greater than 7%, which was similar to the relative availability of medusahead in the community (i.e., 6%). Use of medusahead by ewes was correlated with that observed for

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their lambs ( $r = 0.83$ ;  $P < 0.05$ ), and weaned lambs showed a similar proportion of grazing events on medusahead to those observed before weaning ( $P > 0.05$ ). These results suggest that mothers influence medusahead use by their offspring. They also suggest that despite the low palatability of medusahead, sheep will not select against medusahead when grazing moderately-infested rangeland. The diversity of the plant community likely contributed to this outcome, which might have also reduced the impact of the supplement on medusahead use by sheep.

## Introduction

Medusahead (*Taeniatherium caput-medusae* subsp. *asperum*) is an Eurasian annual grass introduced into the western United States towards the end of the 19th century (Young, 1992; Davies and Johnson, 2008). Since then, this weed has invaded over 10 million hectares of rangeland in the Pacific Northwest, California, Utah and Nevada (Johnson and Davies, 2012).

Grazing represents a sustainable and low-input method for weed control. In fact, stakeholders, scientists and land managers see high potential for the use of grazing as a tool to control medusahead in western rangelands (James et al., 2015). Nevertheless, livestock have been reported to display remarkably low preference for medusahead during grazing, attributed to the low nutritional value of the weed (Lusk et al., 1961; George et al., 1989; Young, 1992). In turn, medusahead avoidance leads to an increased grazing pressure on palatable native plants, which further reduces animal carrying capacity and contributes to the spread of the weed (Hironaka, 1961; Torrel et al., 1961).

New paradigms on foraging behavior emphasize the importance of positive experiences early in life with the biochemical context (provided by the plant community



or supplements) on preference for target feeds (Villalba et al., 2015). Preference for a particular feed depends not only on its intrinsic (e.g., nutritional, toxicological) properties, but also on the nutritional context where that food is ingested. An instance of this type of phenomenon is called induction effect, which consists of an increased intake of an unpalatable food when it is associated with the ingestion of a preferred food in a sequence familiar to the animal (Flaherty, 1996; Provenza et al., 2003). For instance, Caton and Dhuyvetter (1997) and Garcés-Yépez et al. (1997) reported that concentrates containing highly digestible fiber (i.e., beet pulp) increase intake of low-quality forages by livestock because such supplements maintain a favorable rumen environment. Thus, conditioning animals with appropriate supplemental feeds may lead to a more even utilization of palatable and unpalatable resources in a plant community and as a consequence, to the maintenance of biodiversity in the landscape (Provenza et al., 2003; Baraza et al., 2005).

In addition to the nutritional context, positive experiences early in life with mother can have life-long influences on herbivores by causing neurological, morphological, and physiological changes that influence foraging behavior (Provenza and Balph, 1990; Distel et al., 1994, 1996). Given the appropriate nutritional context at an early stage of development, animals may increase consumption of medusahead and its use may persist throughout the lifetime of the individual and across generations.

We hypothesized that an appropriate nutritional context would enhance intake of and preference for unpalatable weeds like medusahead by herbivores and that the effect would be transmitted from mother to offspring. To test this hypothesis we determined the

influence of an energy-based supplement on use of medusahead by ewes and their lambs and the subsequent use of the weed by weaned lambs.

## Material and Methods

### *Study site*

The experiment was conducted on privately-owned land with medusahead infestation in Mantua, Box Elder County, UT, USA (41° 29'51" N and 111°56'32" W). The ecological site is Mountain Stony Loam (Wadman, 2012), which is located between 1670 and 2560 masl and has slopes between 5 and 70%. The soil is stony, cobbly or gravelly loam textured and permeability is moderate slow to moderate. Cold snowy winters and cool dry summers characterize the climate. The plant community is Mountain Big Sagebrush with introduced non-native plant species (Wadman, 2012). The functional group of grasses is composed mainly by *Achnatherum lettermanii*, *Elymus trachycaulus*, *Poa secunda*, and *Elymus spicatus*. Some representative forbs are *Achillea millefolium*, *Allium acuminatum*, *Astragalus argophyllus*, *Aster occidentalis*, *Balsamorhiza sagittata*, *Lupinus caudatus*, and *Castilleja linariifolia*. The dominant shrubs are *Artemisia tridentata* subsp. *vaseyana*, *Cercocarpus montanus*, and *Purshia tridentata* (Wadman, 2012). Non-native grasses are medusahead and *Bromus tectorum*.

### *Grazing blocks*

In order to evaluate the effect of an energy-rich supplement on use of medusahead by ewes and their lambs, six 0.18 ha blocks, each divided into 2 plots (0.09 ha plot) were marked at the study site. Each plot was delimited using electric fence and a pen of ~3.0

m<sup>2</sup> was assembled outside each plot for overnight enclosure of the animals. Culinary water, salt (White Salt Block, North American Salt Company, Overland Park, KS, USA) and trace-mineralized salt blocks (Morton iOfixt T-M, Chicago, IL, USA) were provided in *ad libitum* amounts inside each pen throughout the study. Three control plots (no grazing) were randomly located between the grazed blocks.

In order to test for the effects of exposure with their mothers to a supplement on medusahead use by weaned lambs, six 0.021 ha blocks, each divided into 2 plots (0.0105 ha plot) were marked on the study site with outside pens ( $\sim 3.0$  m<sup>2</sup>) and fenced as described before. Blocks were assembled 50 m to the northeast of the previously described blocks where ewes grazed with their lambs.

#### *Effect of supplementation on medusahead use by ewes and their lambs*

##### *Experimental design*

The study was conducted according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval # 1551). Thirty-six crossbreed ewes with their lambs (2-3 months of age) were dewormed for internal and external parasites (Ivermectin; 0.2 mg/kg BW) and immunized against clostridial diseases (*Clostridium perfringens* types C and D, and *C. tetani*). Twenty-three ewes had single lambs and 13 ewes had twins.

Sheep grazed an orchardgrass (*Dactylis glomerata*) pasture at the Green Canyon Ecology Center, Utah State University, Logan, UT, during May of 2013. All animals were also fed  $\sim 8$  kg d<sup>-1</sup> of an energy-based supplement from May 7 to May 31, 2013 in

order to familiarize the animals with this feed before they grazed medusahead-infested rangeland. The energy supplement was a mixture of beet pulp, barley, and Ca-propionate (Sigma-Aldrich, St. Louis, MO, USA) at a rate of 66:30:4 (as-fed basis).

The average initial body weight (BW) of ewes and lambs was  $72.4 \pm 8.3$  and  $25.0 \pm 8.9$  kg, respectively. All animals were transported to the study site on June 1, 2013.

Within each of the six 0.18-ha experimental blocks described above, one group of 3 ewes with their lambs ( $3.8 \pm 0.8$  lambs/group) received the supplement (Treatment) and grazed in one of the 0.09-ha plots of the block, whereas another group of 3 ewes and lambs ( $4.3 \pm 1.0$  lambs/group) did not receive supplement (Control) and grazed on the other 0.09-ha plot of the block. Each Treatment group received 2.5 kg of the energy supplement from 0750 to 0830 daily. Supplement refusals were recovered immediately after animals were released to graze and weighed to calculate the average supplement intake by group for 6 days when groups were observed grazing their respective plots using the scan sampling technique (see below). All ewes and their lambs were released to graze their respective plots from 0830 to 1700, when all animals were penned overnight. Sheep grazed for 15 consecutive days (from June 2 to 16, 2013). Medusahead was in the late vegetative stage at the beginning of the experiment and in the late reproductive stage towards the end of the experiment.

#### *Scan sampling*

Behavior of ewes and their lambs was recorded at 5-min intervals from 0830 to 1100 and from 1600 to 1700 using the scan sampling technique (Altman, 1974). Foraging activities involved those events when animals were observed grazing medusahead, annual grasses other than medusahead, bunch grasses, and forbs in the plant community;

additional behaviors were: walking, resting, ruminating, nursing, and drinking.

Observations were made in 3 blocks per day during alternate days: 3 randomly selected blocks were scanned by 3 observers on days 1, 3, 6, 8, 11, and 13, whereas the remaining 3 blocks were scanned on days 2, 4, 7, 9, 12, and 14. The proportion of grazing events on medusahead and other functional groups (other annual grasses, bunch grasses, and forbs) in the plant community relative to the total number of scans recorded per day (i.e., during 3.5 h) was determined. Additionally, the scans recorded for each of the 3.5 h periods were divided into 7 intervals of 30 min each, 5 intervals from 0830 to 1100 and 2 intervals from 1600 to 1700. The proportion of grazing events within each interval was calculated as described before.

#### *Evaluation of the plant community*

Plant biomass production was estimated in all grazed and ungrazed plots using a rising plate meter (Michell, 1982). Twenty-five readings of plant height were recorded along a zig-zag transect per plot pre-(June 1, 2013) and post-(June 17, 2013) grazing using a rising plate with an area of 0.0985 m<sup>2</sup>. Plant dry matter (DM) in each plot for calibration curves was assessed pre- and post-grazing by taking 24 random readings of the rising plate meter (Michell, 1982) along with DM production values.

The relative fresh biomass abundance of medusahead and of different plant functional groups (forbs, bunch grasses, and annual grasses other than medusahead) were visually estimated pre- and post-grazing in 25 squares (square area = 0.0985 m<sup>2</sup>) randomly distributed within a zig-zag transect. These visual estimations were made in a randomly selected plot per block with the restriction that 3 plots were grazed by supplemented sheep and 3 plots were grazed by non-supplemented sheep. Shrubs

occurred in low frequency in all plots ( $< 2\%$  of frequency) and their abundance was thus not considered in the study.

Four squares (square area =  $0.0985 \text{ m}^2$ ) within the aforementioned transect were randomly selected and harvested at the ground level. Plant material was taken to the laboratory, manually sorted into medusahead and plant functional groups and weighed on a wet basis (WB). This information was used to calculate the percentage of medusahead and functional groups present in each sample and these values were compared with the relative abundance estimated visually in the plots.

Samples of supplement, medusahead, and functional groups were dried in a forced air oven at  $60^\circ\text{C}$  until constant weight to estimate DM content. A composite of medusahead and each plant functional group were formed with samples from 2 blocks. Samples were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) with a 1-mm screen for chemical analyses.

Available biomass pre- and post-grazing per plot was calculated for medusahead and functional groups as: available biomass (kg DM) = (relative fresh biomass abundance of medusahead or functional group [%] x biomass on WB [kg] per plot / 100) x % DM of medusahead or functional group / 100. By subtracting the available DM biomass post-grazing to the available DM biomass pre-grazing, it was possible to estimate the amount of biomass removed by sheep from each plot on a DM basis (total, medusahead, and functional groups).

The percentage of relative frequency of medusahead and functional groups was estimated per plot as: relative frequency (%) = number of squares with medusahead or functional groups/total number of squares x 100.

### *Medusahead defoliation*

Squares (square area = 90.2 cm<sup>2</sup>) containing medusahead tillers on each grazed and ungrazed plot were marked using flagging tape, which was anchored to the soil with nails at each corner of the square. This procedure is a modification of the technique described by O'Reagain and Grau (1995) to assess defoliation on bunch grasses. A numbered flag was inserted into the soil about 40 cm from each square to target the area and reduce possible cueing effects on grazing behavior. The number of squares selected was similar between plots within the same block. However, the number of marked squares across blocks varied between 5 and 15, as it was proportional to the spatial distribution and abundance of medusahead within each of the blocks.

Medusahead cover in each square was ranked from 1 to 5 according to the percentage of soil covered by medusahead tillers: 1 = 10% cover (9.0 cm<sup>2</sup>), 2 = 30% cover (27.1 cm<sup>2</sup>), 3 = 50% cover (45.1 cm<sup>2</sup>), 4 = 70% cover (63.2 cm<sup>2</sup>), and 5 = 90% cover (81.2 cm<sup>2</sup>). Medusahead tillers within each square were similar in height and the height of the tallest leaf in the tillers was measured in cm. The estimated area of tillers (cm<sup>2</sup>) multiplied by the height of the tallest leaf (cm) was used to estimate the initial volume of the biomass contained inside the marked squares before grazing.

Defoliation of medusahead tillers was measured on days 5, 10, and 15 of the experiment. The proportion of undefoliated tiller area inside each marked square was visually estimated and transformed to area: undefoliated tiller area (cm<sup>2</sup>) = proportion of undefoliated tiller area x area of medusahead tillers (cm<sup>2</sup>). This area was multiplied by the height (cm) of the tallest leaf to estimate the volume of biomass (cm<sup>3</sup>) remaining after grazing. The area of defoliated tillers inside the marked square (defoliated tiller area

$[\text{cm}^2] = \text{total tiller area } [\text{cm}^2] - \text{undefoliated tiller area } [\text{cm}^2])$  was multiplied by the average height (cm) of the leaves remaining on the tillers to estimate the volume of biomass ( $\text{cm}^3$ ) of the grazed tillers. The proportion of medusahead volume removed by sheep was calculated as:  $(\text{initial volume } [\text{cm}^3] - \text{residual volume after grazing } [\text{cm}^3]) / \text{initial volume } (\text{cm}^3)$ . The height of medusahead plants that did not receive defoliation after grazing was used to correct for the initial volume of medusahead biomass since medusahead plants grew in height during grazing.

At the end of the experiment, medusahead tillers in the marked squares within the ungrazed plots were cut at the ground level, taken to the laboratory, and dried to constant weight at 60° C. These biomass values (DM basis) were used to run a linear regression between medusahead tiller volume and DM content. Additionally, the number of medusahead tillers inside each square was counted to run a linear regression between number of tillers and rank (from 1 to 5) assigned to each of the medusahead squares.

#### *Effect of supplementation on medusahead use after weaning*

Lambs were weaned on June 17 and kept in the same groups that grazed with their mothers. Groups of lambs were housed in their respective pens ( $\sim 3.0 \text{ m}^2$ ) outside their grazing plots and fed *ad libitum* amounts of alfalfa pellets for 4 days. Ewes were moved back to the Green Canyon Ecology Center and weighed to calculate the average daily BW change.

Animals grazed their respective 0.0105 ha plots (see grazing blocks) without supplementation from 0830 to 1700 for 3 consecutive days (June 21 to 23). Lambs depleted > 90% of the available plant biomass in all plots after 3 days of grazing and at



this time medusahead was in the late reproductive stage. Thus, animals were removed from the plots after 3 days of grazing.

Lambs' behavior was recorded at 5-min intervals by 3 observers from 0830 to 1100 and from 1600 to 1700 (Altman, 1974) on 2 consecutive days (June 21 and 22). Three randomly selected blocks were scanned each day by the observers. Foraging activities involved those events when animals were observed grazing medusahead, annual grasses other than medusahead, bunch grasses, and forbs in the plant community; other activities recorded were: walking, resting, ruminating, and drinking. The proportion of grazing events on medusahead and other functional groups in the plant community relative to the total number of scans recorded per day (3.5 h) was determined.

The biomass production was estimated pre- and post-grazing using a rising plate meter with a plate area of 0.0985 m<sup>2</sup> (Michell, 1982). Forty readings were randomly taken in treatment and control blocks pre- and post-grazing. Seven random readings of the rising plate meter were recorded before grazing along with DM production values to produce a calibration curve (Michell, 1982). The relative abundance of medusahead and plant functional groups were estimated in each plot by visual inspection of the whole plot by two observers.

Four squares (square area = 90.2 cm<sup>2</sup>) containing medusahead tillers were marked and assessed before and after grazing in each plot as described before to estimate medusahead defoliation. Lambs were moved to the Green Canyon Ecology Center on June 24 and weighed to calculate the average daily BW change.

### *Chemical analyses*

The energy supplement and spatial replicates of medusahead, annual grasses other than medusahead, bunch grasses, and forbs pre- and post-grazing were analyzed for crude protein (CP), neutral (NDF) and acid (ADF) detergent fiber, ash, and acid insoluble ash (AIA). CP was calculated by measuring nitrogen content (Wiles et al., 1998) and then by multiplying this content by 6.25. NDF and ADF were measured according to Van Soest et al. (1991); ADF measure was sequential of NDF. Ash content was obtained by burning samples at 550°C for 6 h (Allen, 1989). Acid insoluble ash (AIA) is an approximation of silica content (i.e., >90% of AIA is silica [Charca et al., 2007]) and it was determined by the method of 2N HCl (Van Keulen and Young, 1977).

### *Statistical analyses*

Analyses were computed using SAS (SAS Inst., Inc. Cary, NC; Version 9.1 for Windows). The covariance matrix structure used was the one that yielded the lowest Akaike information criterion. The model diagnostics included testing for a normal distribution and homoscedasticity. Data were transformed when needed according to the Box-Cox method but non-transformed means  $\pm$  SEM are reported. Means were analyzed using Tukey's multiple comparison tests when  $F$ -ratios were significant ( $P < 0.05$ ). A tendency was considered when  $0.05 < P < 0.15$ .

The correlation coefficient between relative abundance of medusahead and plant functional groups determined by visual estimation and by weight on a WB was determined. From marked medusahead squares into the Control plots, linear regressions were estimated between ranks (1 to 5; independent variable) and number of medusahead tillers (dependent variable), and between volume of medusahead tillers (cm<sup>3</sup>; independent

variable) and medusahead biomass (g DM; dependent variable). These regressions were used as calibration curves to estimate the number of medusahead tillers and biomass within the marked medusahead squares in the grazed plots.

The proportion of events recorded for total grazing events, and for eating medusahead, annual grasses, bunch grasses, and forbs across the 3.5 h of daily observations, and proportion of medusahead volume removed during grazing by ewes with their lambs were analyzed as a mixed-model effects with block as random effect and animal (ewe, lamb), supplement (yes, no) and day as fixed factors. The same model was used to analyze the average proportion of recorded grazing events for ewes and their lambs across successive 30 min intervals during the 3.5 h of daily observations, with the addition of interval as a fixed factor.

For weaned lambs, block was the random effect and previous exposure to supplement (yes, no) was the fixed factor. In order to assess differences in medusahead use by lambs before and after weaning, the proportion of events recorded for grazing medusahead by lambs (before and after weaning) was analyzed using a one-way analysis of variance with day as the fixed factor.

The linear relationship between ewes and their respective lambs for the proportion of grazing events (total grazing events, and grazing events recorded on medusahead, other annual grasses, bunch grasses, and forbs) was estimated by the coefficient of correlation for those events, where each ewe and its offspring (an average of grazing events for twins) were considered a replicate.

Average daily weight gain for ewes and lambs was analyzed as a one-way analysis of covariance with supplement (yes, no) as a fixed factor and the initial body weight as a

covariate. The biomass (DM) removed during grazing by supplemented and non-supplemented sheep was analyzed as a one-way analysis of covariance, with block as covariate and supplement (yes, no) as fixed factors.

Supplement intake by group was analyzed using a one-way analysis of variance with day as the main factor. Content of CP, NDF, ADF, ash, and AIA in the forages was analyzed using a one-way analysis of variance with forage as the main factor.

## Results

### *Effect of supplement on medusahead use by ewes and their lambs*

### *Chemical composition of the plant community*

Medusahead and plant functional groups showed differences in their chemical composition at the beginning of the grazing period ( $P \leq 0.002$ ; Table 2-1). Medusahead showed greater content of CP ( $P \leq 0.0278$ ), ash ( $P \leq 0.0069$ ) and AIA ( $P \leq 0.0008$ ), and lower content of NDF ( $P \leq 0.0008$ ) and ADF ( $P \leq 0.0014$ ) than other annual and bunch grasses. Forbs and medusahead had similar content of CP ( $P = 0.6521$ ) and ash ( $P = 0.3234$ ), but forbs had the lowest content of NDF ( $P < 0.0001$ ) and AIA ( $P = 0.0019$ ) of the forages assayed (Table 2-1).

Towards the end of the grazing period, medusahead and other annual grasses had greater content of CP and lower content of NDF and ADF than bunch grasses. Forbs showed the lowest content of CP, NDF, ADF and AIA at the end of the grazing period, but high concentrations of ash, similar to those values found for medusahead (Table 2-1).

Forages had greater content of CP and lower content of fiber (NDF and ADF) at the beginning than at the end of grazing. Medusahead and forbs had greater content of

ash at the beginning than at the end of the study. Concentration of AIA increased for medusahead as the season progressed, whereas the opposite pattern was observed for forbs (Table 2-1).

### *Supplement intake*

Supplemented groups ate different amounts of supplement across days of scan sampling ( $P < 0.0001$ ). Intake decreased from day 1 to day 2 ( $P = 0.0068$ ), followed by a gradual increment until day 4, which was similar to days 5 and 6 ( $P > 0.05$ ) (Fig. 2-1).

### *Scan sampling*

Supplementation did not have an effect on the proportion of total grazing events recorded or on the use of medusahead or other functional plant groups in the plant community ( $P > 0.05$ ; Fig. 2-2).

Ewes and lambs did not differ in the average proportion of total grazing events ( $P = 0.7019$ ), but they showed different proportion of grazing events across days ( $P < 0.0001$ ), as a greater proportion of grazing events was recorded on days 1, 4, and 6 than on day 2 ( $P < 0.0001$ ); more events were observed on day 4 than on days 3 and 5 ( $P \leq 0.0159$ ), and a tendency for more grazing events was noted on day 6 than on days 3 ( $P = 0.0515$ ) and 5 ( $P = 0.0559$ ) (Fig. 2-2A).

When comparing mothers and their offspring, ewes showed a greater proportion of scans on grazing forbs than those observed in lambs:  $0.15 \pm 0.02$  vs.  $0.11 \pm 0.02$ , respectively ( $P = 0.0281$ ). In contrast, lambs tended to display a greater proportion of grazing events on bunch grasses than ewes  $0.42 \pm 0.03$  vs.  $0.38 \pm 0.04$ , respectively ( $P =$

0.0731). No differences between ewes and lambs were detected for the proportion of events recorded for eating medusahead or other annual grasses ( $P > 0.05$ ).

Ewes and lambs showed a greater proportion of events recorded for eating bunch grasses on day 1 than on days 2, 3, 5, and 6 ( $P < 0.05$ ), and on day 4 than on days 2 and 5 ( $P \leq 0.0191$ ) (Fig. 2-2B). Scans on forbs were lower for days 1 and 2 than for days 4, 5, and 6 ( $P \leq 0.005$ ) (Fig. 2-2C). The proportion of events recorded for grazing medusahead increased across time ( $P < 0.0001$ ), as animals showed a lower proportion of grazing events on medusahead during the first 2 days than during the last 3 days of the grazing period ( $P \leq 0.0387$ ) (Fig. 2-2D).

Annual grasses other than medusahead tended to be utilized to a greater extent during the first days than towards the end of the trial ( $P = 0.099$ ), from an average daily proportion of  $0.014 \pm 0.005$  to  $0.05 \pm 0.002$ . Interactions between factors did not have an effect on grazing events (animal x day, animal x supplement, day x supplement, and animal x day x supplement;  $P > 0.05$ ).

Analyses for the proportion of grazing events recorded across 30 min intervals during the daily 3.5 h of scan sampling showed that supplement did not have effect on the proportion of total grazing events or on the events recorded for grazing medusahead, other annual grasses, or forbs across the day ( $P > 0.05$ ). However, supplemented ewes and their offspring tended to show a lower proportion of grazing events on bunch grasses ( $0.34 \pm 0.05$ ) than non-supplemented ewes and their offspring ( $0.40 \pm 0.06$ ;  $P = 0.1212$ ). The proportion of total grazing events and of events recorded for grazing medusahead, bunch grasses, and forbs varied throughout the day ( $P < 0.0001$ ) (Fig. 2-3). The proportions of total grazing events and of grazing events on bunch grasses and forbs

declined gradually during the first part of the morning scans (i.e., from 0830 to 1030;  $P < 0.05$ ) (Figs. 2-3A to 2-3C). The first part of the afternoon scans (i.e., from 1600 to 1630) showed that all sheep increased the proportion of total grazing events and of grazing events recorded on bunch grasses relative to the last interval recorded in the morning (i.e., from 1030 to 1100;  $P < 0.05$ ) (Figs. 2-3A and 2-3B). The proportion of grazing events on forbs remained relatively constant from 1000 to 1100 and from 1600 to 1700 ( $P > 0.05$ ; Fig. 2-3C). The proportion of events recorded for grazing medusahead was similar during the first part of the morning scans (i.e., from 0830 to 1000) and afternoon scans (i.e., from 1600 to 1630) ( $P > 0.05$ ), but greater than the proportion recorded for the 1030 to 1100 interval ( $P < 0.05$ ) (Fig. 2-3C).

In general, ewes and their lambs showed a significant correlation regarding use of the different functional groups in the plant community and for some categories the magnitude of the correlation was influenced by supplementation (Table 2-2). For instance, non-supplemented mothers and their offspring showed a greater correlation for the proportion of total grazing events and for eating bunch grasses ( $r = 0.72$  and  $r = 0.70$ , respectively) than supplemented sheep ( $r = 0.58$  and  $r = 0.62$ , respectively) ( $P \leq 0.026$ ; Table 2-2). The correlation between mothers and their offspring for grazing medusahead was high and similar for supplemented and non-supplemented animals ( $r = 0.83$ ;  $P < 0.0001$ ; Table 2-2). Supplemented ewes and their lambs showed a significant correlation for the proportion of grazing events recorded on annual grasses ( $r = 0.62$ ;  $P = 0.0059$ ; Table 2-2), but no correlation was detected for non-supplemented sheep ( $r = 0.24$ ;  $P = 0.3369$ ; Table 2-2). Likewise, no correlation between mothers and their offspring

(supplemented [ $r = 0.33$ ] or not-supplemented [ $r = -0.08$ ]) was detected for the use of forbs ( $P > 0.05$ ; Table 2-2).

#### *Evaluation of the plant community*

The average available biomass in the plots grazed by supplemented ewes and their lambs before and after grazing was  $321.7 \pm 18.3$  kg DM plot and  $132.0 \pm 30.9$  kg DM plot, respectively. Thus, the residual biomass represented  $41.1 \pm 9.5\%$  of the initial biomass available. For the plots grazed by non-supplemented ewes and their lambs, the average available biomass pre- and post-grazing was  $289.4 \pm 5.4$  kg DM plot and  $139.3 \pm 22.9$  kg DM plot, respectively. Thus, the residual biomass represented  $40.9 \pm 7.1\%$  of the initial biomass available. Consistent with scan sampling data, the amount of biomass removed by different groups of ewes and lambs was not affected by supplementation ( $P = 0.3503$ ). Biomass production in the ungrazed plots increased from  $282.5 \pm 6.0$  kg DM plot to  $496.2 \pm 55.3$ , which represented a  $76.4 \pm 22.6\%$  increment from the beginning to the end of the trial.

The percentages of relative abundance of medusahead, other annual grasses, bunch grasses and forbs estimated visually in the plots ( $11.0 \pm 3.4\%$ ,  $5.2 \pm 1.8\%$ ,  $22.7 \pm 3.8\%$ , and  $61.0 \pm 4.6\%$  WB, respectively) vs. percentages of relative abundance estimated by weight ( $11.5 \pm 3.7\%$ ,  $3.8 \pm 1.1\%$ ,  $26.2 \pm 4.0\%$ , and  $58.5 \pm 4.8\%$  WB, respectively) showed the following correlation coefficients ( $P < 0.0001$ ): 0.92, 0.74, 0.84, and 0.85, respectively.

The relative abundance of medusahead and plant functional groups in the plant community changed from before to after grazing (Table 2-3). Annual and bunch grasses



decreased in relative abundance after grazing, whereas the opposite pattern occurred for forbs and medusahead.

Based on the composition of the removed biomass in the plots (Table 2-3), the diet of supplemented sheep was composed of  $4.0 \pm 1.9\%$  medusahead,  $9.0 \pm 0.9\%$  annual grasses,  $39.0 \pm 4.6\%$  bunch grasses, and  $48.1 \pm 3.4\%$  forbs; whereas the diet of non-supplemented sheep was composed of  $2.3 \pm 1.1\%$  medusahead,  $25.7 \pm 6.3\%$  annual grasses,  $41.5 \pm 4.9\%$  bunch grasses, and  $30.6 \pm 3.9\%$  forbs.

During the grazing period, ewes and their lambs harvested different percentages of the initial biomass available in the plots (DM basis):  $< 50\%$  of medusahead,  $\sim 15\%$  of other annual grasses,  $< 33\%$  of bunch grasses, and  $< 53\%$  of forbs (Table 2-3). The relative frequency of medusahead increased throughout grazing period, being the opposite for the rest of the functional plant groups (Table 2-3).

#### *Medusahead defoliation*

Medusahead squares ( $90.2 \text{ cm}^2$ ) had an average rank of  $2.4 \pm 0.1$  in a scale of 1 to 5 that represented an area of  $34.3 \pm 2.1 \text{ cm}^2$ . The linear regression between ranks assigned visually and the numbers of medusahead tillers counted in marked medusahead squares of ungrazed plots was: number of tillers =  $1.9158 (\sqrt{\text{rank}}) + 2.77839$ ;  $R^2 = 0.5938$ ;  $P < 0.0001$ . This relationship estimated that the medusahead squares marked into the grazed plots had on average  $54 \pm 9$  tillers per square.

Tillers had an initial height of  $14.4 \pm 0.3 \text{ cm}$  and the average initial volume in the medusahead squares was  $520 \pm 40 \text{ cm}^3$ . The linear regression between the volume ( $\text{cm}^3$ ) of medusahead biomass in each square and the DM content was: DM biomass =

$933.94341 [\text{volume, cm}^3] + 0.83158; R^2 = 0.5936; P < 0.0001$ ). According to this regression and using the initial volume (calculated by multiplying the average tillers' height by their average area), the initial medusahead biomass inside each square was  $1.31 \pm 0.86$  g DM.

Supplementation had no effect on defoliation of medusahead tillers ( $P = 0.3054$ ). However, sheep defoliated different proportions of medusahead across time ( $P = 0.0013$ ; Fig. 2-4); they removed more medusahead on days 5 and 10 than on day 15 ( $P \leq 0.0260$ ; Fig. 2-4). No other effect was observed (supplement x day;  $P = 0.5716$ ).

#### *Effect of supplementation on medusahead use after weaning*

##### *Scan sampling*

Lambs previously supplemented with their mothers tended to show a lower proportion of total grazing events than Control lambs ( $P = 0.0928$ ; Fig. 2-5). Similar to when lambs grazed with their mothers, supplementation did not have an effect on grazing events recorded on medusahead or on other functional groups in the plant community ( $P > 0.05$ ; Fig. 2-5). Moreover, there was not difference in the proportion of grazing events on medusahead when lambs grazed with or without their mothers ( $P > 0.05$ ).

##### *Evaluation of the plant community*

The available biomass pre-grazing was  $3,295 \text{ kg DM ha}^{-1}$  ( $34.6 \text{ kg DM per plot}$ ). After grazing, the residual biomass was  $200 \text{ kg DM ha}^{-1}$  ( $2.1 \text{ kg DM per plot}$ ). The average relative abundance of plants for pre-grazing was:  $19.9 \pm 2.3\%$  medusahead,  $13.3 \pm 1.5\%$  other annual grasses,  $36.1 \pm 2.4\%$  bunch grasses, and  $30.8 \pm 2.4\%$  forbs. The average relative abundance of plants post-grazing was:  $37.1 \pm 4.9\%$  medusahead,  $4.3 \pm$

0.7% other annual grasses,  $23.0 \pm 3.1\%$  bunch grasses, and  $35.5 \pm 6.1\%$  forbs. Thus, forbs maintained their relative abundance, bunch and annual grasses reduced their relative abundance, and medusahead increased its relative abundance after grazing.

#### *Medusahead defoliation*

The marked squares of medusahead tillers had an average rank of  $1.4 \pm 0.1$  in a scale of 1 to 5 that represented  $15.0 \pm 2.0 \text{ cm}^2$ , with an average of  $29 \pm 9$  tillers per square – estimated by the aforementioned relationship between rank and number of tillers- and an average height of  $18.6 \pm 0.5 \text{ cm}$ . The average volume of medusahead tillers was  $300.0 \pm 40.0 \text{ cm}^3$  which represented  $1.11 \pm 0.86 \text{ g DM}$  – estimated by the aforementioned relationship between volume and DM. Weaned lambs with prior exposure to the supplement tended to defoliate a lower proportion of medusahead plants than Control lambs ( $P = 0.1379$ ),  $0.08 \pm 0.03$  vs.  $0.26 \pm 0.09$ , respectively.

#### *Average daily body weight change*

Ewes lost weight during grazing but supplementation did not affect this variable ( $P = 0.9537$ ); supplemented ewes lost  $161.1 \pm 106.7 \text{ g d}^{-1}$  and non-supplemented ewes lost  $224.1 \pm 124.4 \text{ g d}^{-1}$ . Likewise, supplementation did not influence the average daily BW gain for lambs ( $P = 0.2204$ ); supplemented lambs gained  $150.2 \pm 21.2 \text{ g d}^{-1}$  and non-supplemented lambs gained  $117.8 \pm 15.9 \text{ g d}^{-1}$ .

## Discussion

### *Medusahead and ewe-lamb supplementation*

A recent study suggests that energy-dense supplements increase use of medusahead by lambs relative to non-supplemented animals (Hamilton et al., 2015). Nevertheless, the increase reported in that study was modest and substantially below the intake capacity of the animals under study. A dietary ingredient, Ca-propionate, was added to the supplement used in the present study with the aim of further enhancing medusahead intake and animal performance relative to other supplements previously assayed. Propionate supplementation may improve ruminant nutrition when glucogenic precursors are inadequate due to low-quality diets and/or increased energy demands (Mulliniks et al., 2011). Despite this addition, the supplement used in this study did not have an effect on medusahead use as estimated by the scan sampling technique and by the volume of defoliated medusahead tillers. Consistent with these results and under more controlled experimental conditions, i.e., by offering weighed amounts of medusahead to sheep in pens, Villalba and Burritt (2015) did not find a positive effect of energy supplements containing Ca propionate on intake of medusahead by lambs.

In addition to a lack of supplement effects, a lower number of grazing events were recorded for medusahead than for bunch grasses, even when medusahead showed a greater content of CP and lower concentration of fiber than did bunch grasses. However, medusahead had 61% more silica (estimated by AIA) than bunch grasses at the beginning of the study. Silica decreases preference for grasses in rabbits, voles, and sheep when its concentration increases in the plants' tissues (Massey and Hartley, 2006; Cotterill et al., 2007; Massey et al., 2009). Amorphous silica is a structural component of the grass

epidermis, representing a physical barrier (Mayland and Shewmaker, 2001) against breakup of plant tissues and thus a restriction for the release of nutrients to the rumen environment (Bae et al., 1997; Hunt et al., 2008). This constraint decreases the rate of fermentation as observed in *in vitro* digestibility studies (Chapter 4). Thus, it is likely that due to this blocking action of silica on forage digestion, the influence of supplements at enhancing use of medusahead by ruminants are attenuated or prevented as observed in this and previous studies.

#### *Use of medusahead relative to its abundance*

Results from this study also show that ewes and their lambs displayed low levels of medusahead use during grazing. Animals spent an average of ~5% of the activities recorded during scan sampling grazing medusahead. Consistent with this finding, Yiakoulaki et al. (2009) reported that *T. caput-medusae* (unknown subsp.) represented 6.4% of the diet in adult ewes grazing in the natural habitat of this grass (i.e., Greece).

The estimation of biomass harvested by sheep in this study showed that medusahead represented approximately 3% of the ewes and lambs' diet, below the 5% average recorded during scan sampling. The former may represent an underestimation as medusahead might have increased in frequency during the grazing period (June 2 to June 16 of 2013), a pattern that was observed in June of 2014 (Chapter 3). This increase in medusahead frequency during grazing may be attributed to the fact that medusahead grows 2 weeks later than native plants (Young, 1992).

Collectively, our results suggest that when the relative abundance of medusahead in a plant community is below a certain threshold (i.e., 6% in this study), animals may not

select against this weed despite its low palatability. In contrast, when sheep are exposed to *ad libitum* amounts of medusahead and then to a basal diet of grass hay, the proportion of medusahead in the diet declines drastically, indicating in these conditions a clear state of avoidance (Hamilton et al., 2015; Villalba and Burritt, 2015).

Herbivores exposed too frequently or to too large quantity of a specific feed will satiate on that feed, decreasing their preference relative to an alternative feed (Provenza, 1996). Satiation may create mild to strong states of feed aversion that are more pronounced when feeds are nutritionally imbalanced or when they contain toxins (Provenza, 1996). Medusahead ingestion could lead to nutrient imbalances as its digestion is impaired by the antinutritional factor silica as described above which may induce a strong state of avoidance when exposure to the weed is high (i.e., herbivores grazing medusahead monocultures). In contrast, when the abundance of medusahead is low, the negative post-ingestive consequences of the weed (e.g., poor digestibility, low release of nutrients) may be diluted and thus experienced to a lesser extent by the herbivore. In addition, the ingestion of additional nutritious plants from the community may attenuate the typical aversive responses to medusahead observed when the weed is abundant. It is likely that the diversity of plants available in the plots of this study provided the appropriate nutritional context to at least prevent the avoidance of the weed, an effect which in turn reduced the potential positive impact of the supplement on medusahead use by ewes and their lambs.

#### *Use of medusahead across days*

Ewes and their lambs displayed more foraging events on medusahead towards the second half of the grazing period, even when medusahead was at mid-late reproductive

stage with lower CP content and greater AIA content than when the weed was less mature - at the beginning of the grazing period. This foraging pattern could be explained by the fact that animals became more familiar with the weed because familiarity with a certain feed increases preference (Provenza, 1995). Alternatively, the presence of inflorescences in more mature plants may have contributed to an increased intake as sheep show greater preferences for medusahead inflorescences than for leaves and stems (Villalba and Burritt, 2015). Preference for medusahead inflorescences could be due to the greater DM digestibility (68.0%) and to the lower concentration of AIA (7.7%) for this plant component than for stems and leaves (57.8% of DM digestibility and 9.3% of AIA). Additionally, stems may represent 50% of whole plant (Chapter 4).

Despite the greater use of medusahead towards the end of the grazing trial revealed during scan sampling, this pattern was not reflected in the proportion of defoliated medusahead tillers recorded in the vegetation during that period (i.e., from day 10 to day 15). In fact, the proportion of defoliation was lower in the marked medusahead squares for day 15 than for day 10. It is possible that the depletion of biomass (and thus inflorescences) in the small squares attenuated the effect observed at a larger scale with more ungrazed plants available during scan sampling.

In addition to the different grazing patterns of medusahead across days, scan sampling revealed differences in medusahead use throughout the day. Ewes and their lambs were observed eating medusahead almost twofold more during the first 1.5 h of grazing (from 0830 to 1000) than during the following hour (from 1000 to 1100). Similarly, sheep in pens showed a greater daily use of medusahead during the first hour of a 3-h period of exposure (i.e., from 1000 to 1300; Chapter 3). This suggests that either

during grazing or in confinement sheep display a greater daily use of medusahead during the initial hour of exposure. It is worth mentioning that the nutritional context was different between pen and grazing studies; in the present study, ewes and lambs had available different grasses and forbs species throughout the day, whereas sheep in pens had only *ad libitum* access to medusahead with no alternative feeds or just a restricted amount of alfalfa hay.

Ewes and lambs increased the use of medusahead in the afternoon (from 1600 to 1630) to a similar proportion to that observed in the morning. It is likely that after 6 h of displaying a peak of medusahead use in the morning, the time elapsed was enough to reduce fill effects which allowed animals to consume more medusahead in the afternoon hours. In addition, an effective strategy for ruminants could be to eat a bulky feed rich in fiber with a low rate of passage (such as grasses) in the afternoon, maintaining rumen fill and reducing the need to eat at night, thus reducing the likelihood of predation (Rutter, 2006).

#### *Effects of experience on medusahead use by lambs*

Weaned lambs showed similar grazing patterns before and after weaning, with a greater number of grazing events on bunch grasses than on forbs. Likewise, the proportion of medusahead in the scans recorded by weaned lambs (5%) was similar to that observed towards the end of the grazing period when they grazed with their mothers. Ewes and lambs showed a lower proportion of medusahead scans at the beginning of the trial ( $\leq 3.5\%$ ), which progressed towards the end of the grazing period. Weaned lambs had experience at grazing medusahead with their mothers and such experience likely allowed them to include a greater proportion of medusahead into their diet after weaning.



Ramos and Tennessen (1992) reported that experience with mother increases the initial acceptance of pasture and preference for specific forages. The sensitive period for young herbivores to learn from their mothers is during the transition from monogastric to ruminant digestive physiology; i.e., during weaning (Provenza and Balph, 1987). It is likely that this early exposure with mother allowed lambs to consume medusahead in proportion to its abundance from the first day they grazed as weaned lambs.

Weaned lambs without prior exposure to the supplement tended to defoliate a greater proportion of medusahead than supplemented lambs. Likewise, non-supplemented ewes and their offspring tended to show a greater proportion of events on bunch grasses than non-supplemented ewes and their offspring. It is likely that this pattern influenced medusahead use after weaning. Nevertheless, this effect was minor and scan-sampling results did not reveal supplementation effects on medusahead use by lambs.

#### *Individual variation in the use of medusahead*

Animal-to-animal variability is common within species, breeds, and even for a given herd or flock regarding the propensity to consume certain plants in a community such as woody species (Baraza et al., 2009; Estell et al., 2012). Consistent with this variability, sheep show individual variation with regards to the amount of medusahead they can ingest. For instance, ewes and their lambs showed variation in the proportion of grazing events recorded for medusahead (i.e., 0 to 12%) in this study. Similarly, Hamilton et al. (2015) and Villalba and Burritt (2015) found clear individual variation with regards to medusahead intake by lambs of 2 to 3 months of age.

A significant correlation between mothers and their offspring was observed for medusahead use during scan sampling; the relative extent of medusahead use by mothers

(low, medium or high) was reflected in the use of medusahead by their offspring. Thus, results from this study suggest that individual variation in medusahead use is passed from mother to offspring, either through genetics and/or observational learning. Social models play a key role in diet selection and food preferences of young ruminants (Thorhallsdottir et al., 1987). As offspring begin to forage, they further learn what to eat (e.g., Mirza and Provenza, 1990, 1992; Thorhallsdottir et al., 1990) and where to go (e.g., Howery et al., 1998) from mother. It may be possible to capitalize on this individual variation for targeted grazing treatments by selecting females – either through genetic markers or observation- that show a high propensity to consume medusahead as they most likely will have offspring with similar dietary habits.

#### *Plant community and ewe-lamb supplementation*

Supplementation did not have an effect on the total number of grazing events recorded in the plant community. Similarly, supplementation with corn in the morning did not affect grazing time in steers (Adams, 1985), but supplemented steers consumed approximately 24% less forage per hour than non-supplemented animals. However, consistent with scan sampling data biomass removal estimates in this study did not reveal differences between supplemented and non-supplemented groups.

Supplements formulated with ingredients than contain highly digestible fiber (i.e., barley grain and beet pulp) fed at low levels can increase intake of low-quality forages by livestock (Caton and Dhuyvetter, 1997; Garcés-Yépez et al., 1997), an effect that was not observed in this study. Moore et al. (1999) suggest that it is difficult to predict the effect of supplementation on the voluntary intake of forages, although supplementation in

general favors intake of native forages when these are nitrogen deficient in relation to their energy content.

When ewes grazed with their lambs, a greater number of grazing events was recorded for bunch grasses and the pattern of scans recorded across days was similar to that observed for total grazing events, suggesting that bunch grasses had a strong influence on grazing time. As days elapsed, ewes and their lambs increased the number of grazing events on forbs likely to keep their average intake rate stable as bunch grasses were being depleted (Agreil et al., 2005). Towards the end of the trial, bunch grasses had lower nutrient quality than forbs, but the proportion of scans recorded on grazing forbs was never greater than that recorded for grazing bunch grasses.

Ewes showed a greater proportion of scans on grazing forbs than lambs. In contrast, lambs tended to display a greater proportion of grazing events on bunch grasses than ewes. Forbs had greater content of CP than bunch grasses and it is likely that the extra CP received with milk reduced the need by lambs to consume additional protein from forbs. In contrast, lactating ewes had an increased CP requirement (NRC, 2007), which likely enhanced preference for forbs.

Ruminants partition their grazing time into meals, with longer meals early in the morning and later in the afternoon, with intermittent grazing occurring throughout other periods of the day. Feeding and movements are interrupted in the middle of the day when temperatures are higher (Adams, 1985; Agreil et al., 2005). Such grazing pattern was observed for ewes with their offspring in this study, with greater grazing activity from 0830 to 1000 and from 1600 to 1630.

The supplement tended to reduce the grazing events on bunch grasses, likely as result of substitutive effects that have been observed under barley supplementation with rates  $\geq 0.7\%$  of BW in steers (Caton and Dhuyvetter, 1997), below the level of supplementation used in the present study. Furthermore, substitutive effects are reported for feeds rich in starch and poor in fiber such as corn grain (Matejovsky and Sanson, 1995), associated with an increase in the proportion of propionate at the expense of acetate (France and Dijkstra, 2005). The supplement in this study was not rich in starch but it had Ca-propionate, a glucogenic precursor (Mulliniks et al., 2011), which could have led to a similar effect to that observed for supplements rich in grains.

#### *Animal performance*

Supplementation had no effect on animals' performance; ewes lost similar weight and lambs gained similar weight during grazing. Moore et al. (1999) reported that supplementation does not ensure weight gain in cattle, and in many cases, weight does not increase when forages are supplemented and sometimes it even decreases. Similarly, Caton and Dhuyvetter (1997) found that the majority of data about energy supplementation at pasture suggests that it does not influence production or that it reduces weight and body condition score in cows. The variation in the effect of energy supplementation depends on the chemical characteristics of supplements and forages, amounts, and increments in animal's maintenance needs under grazing.

#### **Implications**

Sheep ate low amounts of the unpalatable weed medusahead and an energy-based supplement did not affect this outcome. Nevertheless, despite its low palatability, sheep

did not select against medusahead when grazing moderately-infested rangeland, a foraging pattern which does not confer a competitive advantage to the weed. Thus, managing grazing in moderately-infested pastures without supplementation may prevent the spread of the weed. Weaned lambs may continue harvesting medusahead at similar proportions to those observed before weaning, without the need to learn from trial and error, thus reducing the period of time needed to ingest medusahead to their capacity. A delay in animals learning to ingest medusahead due to unfamiliarity will make the weed more unpalatable due to maturity and increase the likelihood of dropping viable seeds into the soil. Finally, it may be possible to select reproductive females with greater preference for medusahead and establish homogeneous flocks with greater capacity to harvest medusahead.

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**Table 2-1**

Chemical composition of the energy supplement and of medusahead and functional groups in the plant community where ewes and their lambs grazed during the study.

Feed resource	Content (g kg <sup>-1</sup> dry matter)				
	CP	NDF	ADF	Ash	AIA
Energy supplement <sup>§</sup>	105.9	320.1	166.5	93.1	22.9
Plant community in rangeland plots (mean ± SEM) <sup>†</sup>					
Pre-grazing					
Medusahead	171.9	546.7	247.3	127.3	68.3
	± 8.5 <sup>a</sup>	± 5.6 <sup>a</sup>	± 1.9 <sup>a</sup>	± 2.9 <sup>a</sup>	± 2.0 <sup>a</sup>
Other annual	117.2	595.9	290.8	87.5	40.9
grasses	± 4.9 <sup>b</sup>	± 1.1 <sup>b</sup>	± 2.0 <sup>b</sup>	± 4.8 <sup>bc</sup>	± 1.6 <sup>b</sup>
Bunch grasses	95.3	584.1	288.1	82.8	42.6
	± 1.4 <sup>c</sup>	± 7.8 <sup>b</sup>	± 4.6 <sup>b</sup>	± 3.0 <sup>b</sup>	± 1.2 <sup>b</sup>
Forbs	153.5	345.9	233.5	110.2	18.8
	± 20.2 <sup>ab</sup>	± 14.7 <sup>c</sup>	± 11.6 <sup>a</sup>	± 9.9 <sup>ac</sup>	± 4.6 <sup>c</sup>
Post-grazing					
Medusahead	84.2	653.4	329.6	107.3	78.7
	± 3.5	± 4.0	± 9.7	± 6.0	± 7.4
Other annual	83.5 <sup>§</sup>	655.6 <sup>§</sup>	339.0 <sup>§</sup>	NA	NA
grasses					
Bunch grasses	19.3	718.9	407.1	NA	NA
	± 0.0	± 4.6	± 5.6		
Forbs	9.2	383.3	275.2	98.5	4.9
	± 1.2	± 29.7	± 23.0	± 2.5	± 0.5

CP= crude protein

NDF= neutral detergent fiber

ADF= acid detergent fiber

Ash= mineral fraction

AIA= acid insoluble ash (> 90% is silica; Charca et al., 2007)

NA= no analyzed for insufficient sample

<sup>§</sup> Chemical analyses of 1 replicate

<sup>†</sup> Chemical analyses of 3 spatial replicates

Same superscript into column of pre-grazing means no difference ( $P > 0.05$ )

**Table 2-2**

Minimum and maximum values for the proportion of grazing events recorded on medusahead and functional groups in the plant community by ewes and their offspring and the correlation coefficients ( $r$ ) for those events between ewes and their respective lambs.

Event <sup>1</sup>	Ewes	Lambs	$r$	$P$ -value
Supplemented sheep				
Total grazing events	0.40 - 0.74	0.36 - 0.97	0.58	0.0124
Grazing medusahead	0.00 - 0.12	0.00 - 0.11	0.83	< 0.0001
Grazing other annual grasses	0.00 - 0.03	0.00 - 0.04	0.62	0.0059
Grazing bunch grasses	0.23 - 0.50	0.26 - 0.67	0.52	0.0269
Grazing forbs	0.07 - 0.28	0.06 - 0.25	0.33	0.1762
Non-supplemented sheep				
Total grazing events	0.39 - 0.79	0.47 - 0.93	0.72	0.0008
Grazing medusahead	0.01 - 0.12	0.00 - 0.10	0.83	< 0.0001
Grazing other annual grasses	0.00 - 0.02	0.00 - 0.03	0.24	0.3359
Grazing bunch grasses	0.27 - 0.50	0.31 - 0.69	0.70	0.0013
Grazing forbs	0.07 - 0.20	0.05 - 0.17	-0.08	0.7484

<sup>1</sup> The proportion of grazing events was calculated from observations during 3.5-h of scan sampling (from 0830 to 1100 and from 1600 to 1700) during 6 days.

Eighteen ewes with their offspring were clustered into 6 groups ( $n=3$ ) and they received an energy-rich supplement in the morning (Supplemented ewes grazing with their offspring). A Control group of eighteen ewes clustered into 6 groups ( $n=3$ ) did not receive the supplement (Non-supplemented ewes grazing with their offspring).

**Table 2-3**

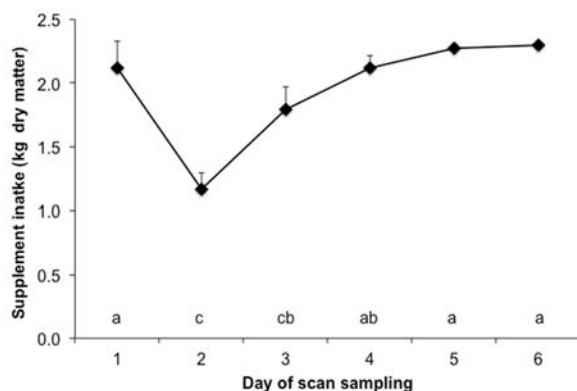
Biomass and relative frequency (mean  $\pm$  SEM) of medusahead and plant functional groups in plots grazed by ewes and their lambs during the study.

		Plant functional groups			
		Medusahead	Other annual grasses	Bunch grasses	Forbs
		Relative abundance <sup>a</sup> , %			
Plots with supplemented sheep					
Pre-grazing	5.8 ± 1.1	5.5 ± 0.4	25.7 ± 2.5	63.0 ± 3.8	
Post-grazing	9.5 ± 4.8	3.4 ± 1.0	20.2 ± 1.9	66.9 ± 5.5	
Plots with non-supplemented sheep					
Pre-grazing	2.7 ± 1.3	14.0 ± 3.2	27.3 ± 0.9	56.1 ± 3.6	
Post-grazing	5.4 ± 3.9	5.7 ± 1.5	20.0 ± 2.0	68.8 ± 7.0	
Harvested biomass by sheep <sup>b</sup> , kg dry matter per plot (0.09 ha)					
Sheep group					
Supplement	11.5 ± 5.4	21.6 ± 1.3	93.4 ± 6.2	120.3 ± 22.3	
No supplement	3.5 ± 2.9	45.1 ± 10.4	72.3 ± 8.3	55.3 ± 13.2	
Harvesting of initial available biomass by sheep, %					
Plot					
Supplement	49.8 ± 25.4	15.6 ± 4.1	23.7 ± 3.3	29.8 ± 6.2	
No supplement	38.5 ± 21.3	15.4 ± 2.6	32.6 ± 6.7	52.7 ± 14.5	
Relative frequency <sup>a</sup> , %					
Plots with supplemented sheep					
Pre-grazing	30.7 ± 4.8	49.3 ± 9.3	90.7 ± 1.3	100.0 ± 0.0	
Post-grazing	48.0 ± 8.0	40.0 ± 0.0	66.7 ± 2.7	92.0 ± 0.0	
Plots with non-supplemented sheep					
Pre-grazing	14.7 ± 5.8	69.3 ± 1.3	94.7 ± 1.3	98.7 ± 1.3	
Post-grazing	22.7 ± 5.8	33.3 ± 5.8	72.0 ± 8.0	88.0 ± 2.3	

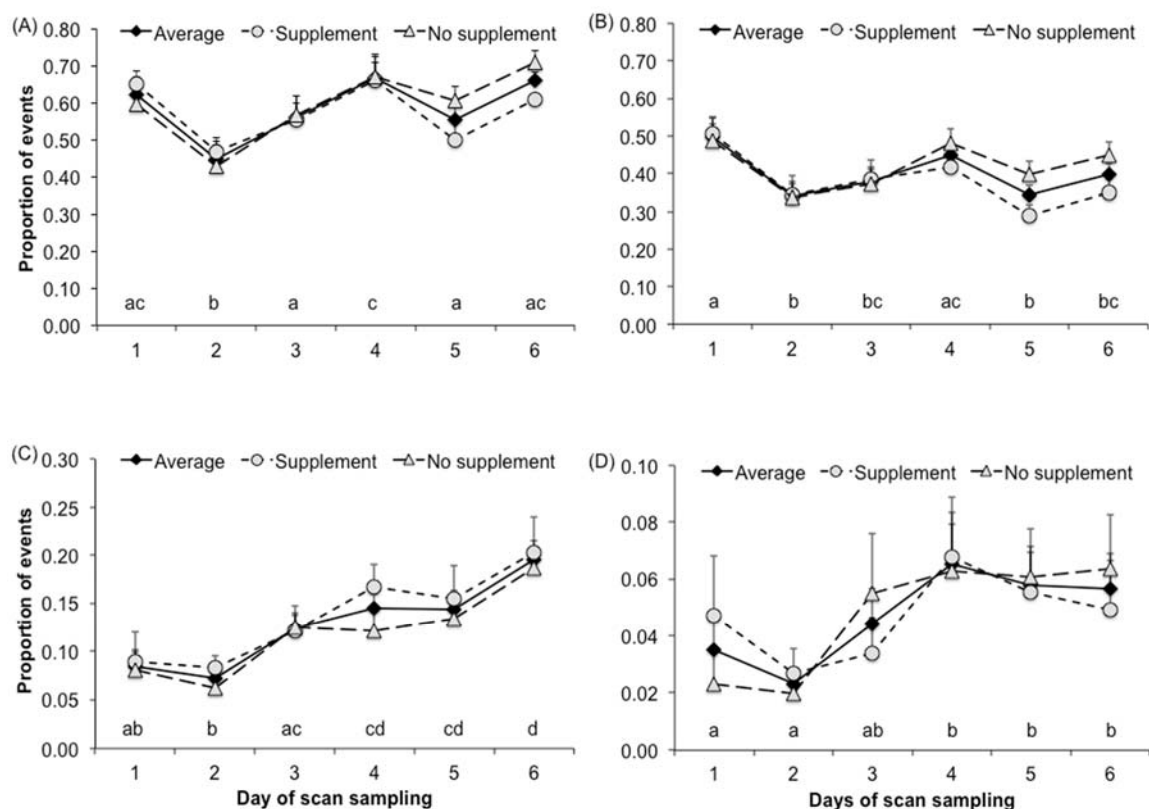
<sup>a</sup> Visually estimated from 1 plot randomly selected from each of the 6 blocks present in

the study, 3 plots were grazed by ewes with their lambs that received an energy supplement (supplemented sheep) and 3 plots were grazed by non-supplemented ewes and their lambs (non-supplemented sheep).

<sup>b</sup> Harvested biomass included biomass removed after grazing due to grazing or trampling.

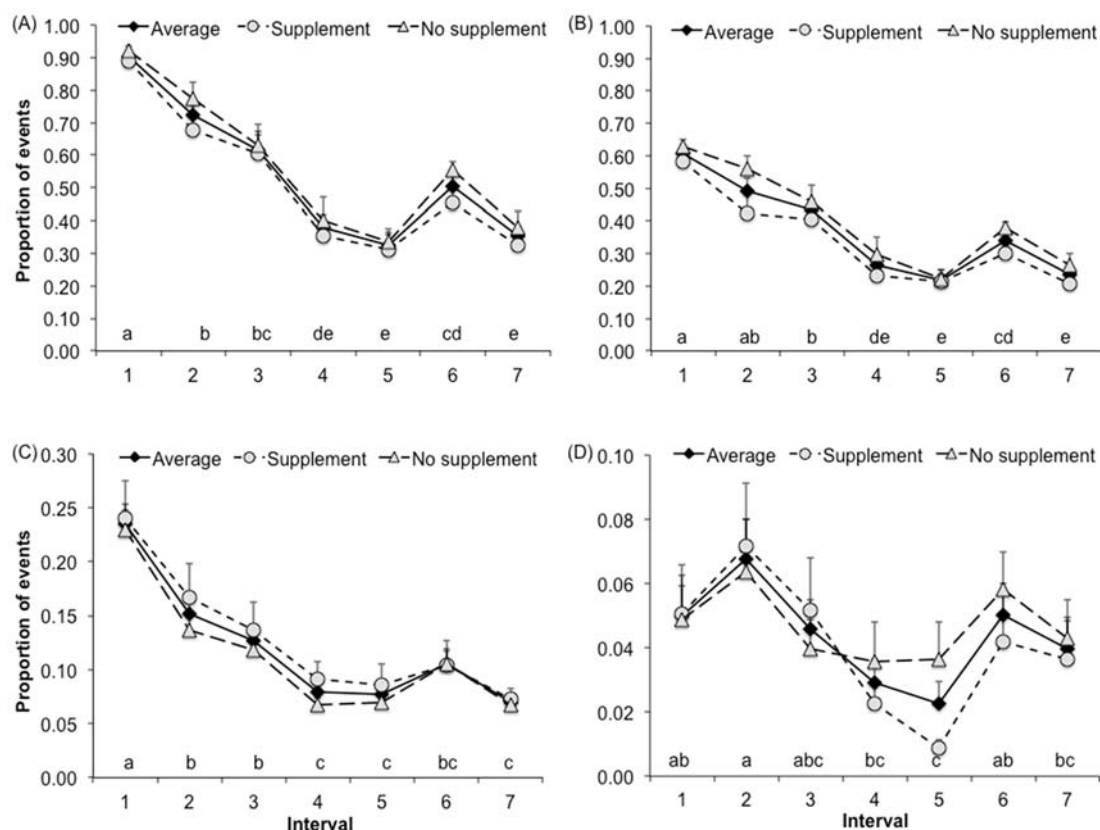


**Figure 2-1.** Average daily intake of an energy supplement by ewes and their lambs for each of the scan sampling days in all groups of the study. The energy supplement was a mixture of beet pulp, barley, and Ca-propionate at a rate of 66:30:4. Six groups of 3 ewes group with their lambs received 2.35 kg dry matter group day<sup>-1</sup> of the supplement (from 0750 to 0830) and then grazed on plots with a moderate level of medusahead infestation from 0830 to 1700. Vertical bars represent the SEM. Means with the same letter did not differ ( $P > 0.05$ ).

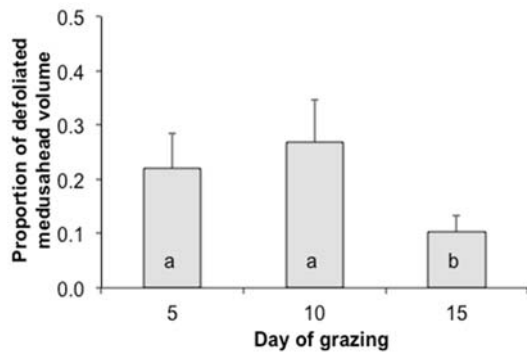


**Figure 2-2.** Proportion of daily grazing events recorded by supplemented and non-supplemented groups (n=6/treatment) of ewes and their lambs and the average for both treatments. Ewes grazed with their lambs on plots with moderate levels of medusahead infestation, grazing events were recorded for 3.5 h (from 0830 to 1100 and from 1600 to 1700). Six groups of 3 ewes with their lambs received an energy-dense supplement before grazing their respective plots (Supplement), whereas six other groups (No Supplement) did not receive the supplement. (A) Proportion of total grazing events; (B) proportion of grazing events on bunch grasses; (C) proportion of grazing events on forbs, and (D) proportion of grazing events on medusahead. Vertical bars represent the SEM. For each day, average proportion of events with the same letter did not differ ( $P > 0.05$ ).

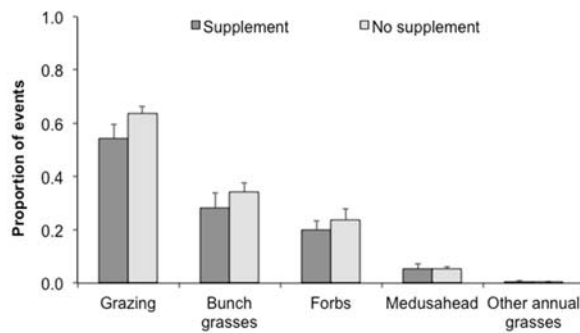




**Figure 2-3.** Proportion of grazing events recorded across 7 time intervals of 30 min each during 3.5 h of scan sampling per day (from 0830 to 1100 and from 1600 to 1700) by ewes and their lambs. Ewes and their lambs grazed in plots with moderate levels of medusahead infestation. Six groups of 3 ewes with their lambs received an energy-dense supplement before grazing their respective plots (Supplement), whereas six other groups (No Supplement) did not receive the supplement. (A) Proportion of total grazing events; (B) proportion of grazing events on bunch grasses; (C) proportion of grazing events on forbs, and (D) proportion of grazing events on medusahead. Vertical bars represent the SEM. For each interval of scan sampling, average proportion of events with the same letter did not differ ( $P > 0.05$ ).



**Figure 2-4.** Average proportion of defoliated medusahead tillers by ewes and their lambs grazing experimental plots with a moderate level of medusahead infestation. Six groups of 3 ewes with their lambs received an energy-dense supplement before grazing their respective plots (Supplement), whereas six other groups (No Supplement) did not receive the supplement. The proportion of defoliated tillers in marked squares (90.2 cm<sup>2</sup>) within each plot was measured every 5 days. No differences between supplemented and no supplemented treatments were detected ( $P > 0.05$ ), thus averages are presented. Vertical bars represent the SEM. Days with the same letter did not differ ( $P > 0.05$ ).



**Figure 2-5.** Average proportion of total grazing events and grazing events on different functional groups recorded during 3.5 h of scan sampling (from 0830 to 1100 and from 1600 to 1700) when 12 groups of weaned lambs grazed experimental plots with moderate level of medusahead infestation. Prior to weaning, six groups of lambs grazed with their mothers but they received an energy-dense supplement before grazing their respective plots (Supplement). Six other groups (No Supplement) did not receive the supplement. Vertical bars are SEM.

CHAPTER 3

EFFECTS OF EARLY EXPERIENCE AND ALTERNATIVE FEEDS ON  
MEDUSAHEAD (*TAENIATHERUM CAPUT-MEDUSAE* SSP.  
*ASPERUM*) INTAKE BY SHEEP<sup>1</sup>

**Abstract**

The goals of this study were to test the effects of early experience at grazing medusahead –an invasive unpalatable weed - and the availability of forage alternatives on intake of medusahead later in life by sheep. The study involved two experiments of 10 days each. During the first experiment, yearling sheep were penned individually in a 2x2 factorial design with early experience at grazing medusahead (yes [ $n = 15$ ], no [ $n = 13$ ]) and availability of alfalfa hay (yes, no) as the main factors. All animals were fed *ad libitum* amounts of freshly harvested medusahead daily from 1000 to 1300 and half of the animals in the experienced ( $n = 8$ ) and inexperienced groups ( $n = 7$ ) received alfalfa hay (0.31% body weight). All groups had a basal diet of tall fescue hay. Intake of the forages, NaCl and trace-mineralized salt blocks was measured. Sheep were then clustered into 10 groups of 3 yearlings each ( $n = 5$  early experience;  $n = 5$  experience gained during Experiment 1) and grazed 10 plots of medusahead-infested rangeland. Grazing events were recorded daily at 5 min intervals from 0830 to 1000 and from 1600 to 1700 and defoliation of medusahead tillers was determined every 5 days. During Experiment 1, medusahead intake was not affected by early experience or alfalfa availability ( $P > 0.05$ ) but medusahead intake across days was cyclic with more pronounced intake peaks at the

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beginning and end of the experiment for inexperienced animals ( $P < 0.05$ ). Experienced sheep showed a greater gain-to-feed ratio ( $P < 0.05$ ), and tended to have greater average daily body weight gains and salt intake ( $P < 0.10$ ) than inexperienced sheep. Sheep fed alfalfa tended to eat less trace-mineralized salt blocks than sheep that did not receive this forage ( $P < 0.10$ ). During Experiment 2, the percentage of grazing events recorded on medusahead by both groups of animals was almost nil ( $0.44 \pm 0.22\%$ ), although experienced animals tended to defoliate more medusahead tillers than inexperienced animals ( $P < 0.10$ ). In conclusion, when sheep were fed *ad libitum* amounts of medusahead, intake was low and cyclic with a more even consumption of the weed by experienced animals. Under these conditions, early experience also favored animal performance. However, it is likely that such high exposure to medusahead attenuated the subsequent use of the weed during grazing.

### **Implications**

Intake of the invasive weed medusahead by livestock is low and cyclic. Early experience with grazing medusahead or availability of forage alternatives like alfalfa hay did not enhance medusahead intake later in life by sheep. However, such early experience may lead to a more even utilization of medusahead across days and to improved animal gain-to-feed ratio. High exposure to medusahead in a patch may lead to the subsequent avoidance of the weed when sheep graze in a diverse plant community.

### **Introduction**

Medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) is a Eurasian annual grass that due to its invasiveness and poor nutritional quality negatively impacts the structure and

functioning of rangeland ecosystems (Young, 1992; Davies and Johnson, 2008). The spread of this weed threatens millions of hectares of rangeland in the western United States despite the different methods attempted for its control (Sheley and James, 2010; Davies and Sheley, 2011). Grazing represents a sustainable and successful alternative for the control of invasive plant species (Frost and Launchbaugh, 2003; Vavra *et al.*, 2007), which is typically conducted with domestic sheep and goats. Nevertheless, results regarding the control of medusahead through grazing have been poor and variable (James *et al.*, 2015), an outcome attributed to the low intake and palatability of this weed (Young, 1992). Despite this constraint, stakeholders in some regions of the United States list grazing as a preferred management tool to control medusahead (James *et al.*, 2015).

Foraging decisions by herbivores can be modified by environmental experiences (Provenza and Balph, 1987), which in turn influence animal fitness and in the process, the structure and composition of plant communities (Provenza and Balph, 1990). In this regard, mother represents a key social model, which allows the offspring to better adapt to novel foods and habitats (Provenza and Balph, 1987). For instance, mother increases the efficiency of learning about novel nutritious foods and reduces the risk of overingesting poisonous plants (Provenza, 1996). Young animals learn with their mothers to consume low-quality forages (Distel *et al.*, 1994), as early experiences have life-long influences on herbivores by causing neurological, morphological, and physiological changes (Provenza and Balph, 1990) through changes in gene expression (Fish *et al.*, 2004) which allow for a greater efficiency of nutrient use (Distel *et al.*, 1994; Chadwick *et al.*, 2009).

In addition to the influence of mother, individual experiences with the chemical and physical characteristics of the feed environment have significant impacts on herbivores' foraging decisions (Provenza, 1996; Provenza and Villalba, 2006). For instance, intake of and preference for an unpalatable food can increase when its ingestion is associated with the consumption of a high-quality supplement in a sequence familiar to the animal (Provenza *et al.*, 2003; Baraza *et al.*, 2005). Certain nutrients in the associated plant community or provided by supplements (e.g., carbohydrates, proteins, minerals) have the potential to increase preference for poor-quality forages (Provenza *et al.*, 2003). Thus, experiences early in life with mother and an adequate supply of nutrients may act synergistically to enhance use of unpalatable forages like medusahead by herbivores, an effect which may persist throughout the life of an individual and across generations. In turn, these responses will contribute to a more even utilization of resources in a plant community (e.g., palatable and unpalatable plants) and as a consequence, to the maintenance of biodiversity in the landscape (Provenza *et al.*, 2003).

We hypothesized that herbivores will increase the utilization of unpalatable forages like medusahead when given the appropriate environmental context early in life (i.e., mother, supplemental nutrients). To test this hypothesis we determined the effects of early experience at grazing medusahead and the availability of forage alternatives on intake of medusahead in confinement and during grazing by sheep.

## Materials and Methods

### *Exposure to Medusahead*

Thirty-six crossbred ewes with their lambs (2-3 months of age) grazed medusahead-infested rangeland with a moderate (~10% in the plant community) level of medusahead infestation during June of 2013. Details for this exposure are presented in Chapter 2. Briefly, the foraging behavior of ewes and their lambs grazing medusahead-infested rangeland was recorded at 5-min intervals from 0830 to 1100 and from 1600 to 1700 for 12 days using the scan sampling technique (Altman, 1974). The average percentage of events recorded for lambs eating medusahead was  $4.4 \pm 0.5\%$  of the total observed behavioral events and  $58.0 \pm 3.3\%$  of the total observed behavioral events involved grazing activities.

After exposure to medusahead, 15 randomly-selected lambs (experienced animals) out of a group of 49 were kept in an orchardgrass (*Dactylis glomerata*) pasture from June 24 of 2013 to May 5 of 2014 and fed alfalfa (*Medicago sativa*) hay in *ad libitum* amounts from November 2013 to March 2014. Animals then received a diet of endophyte-free tall fescue (*Festuca arundinacea*) hay from April 1 to May 5 of 2014. Additionally, 15 lambs of similar age (8 months old) without exposure to medusahead (inexperienced animals- Controls) were kept in the same orchardgrass pasture from October 20, 2013 to May 5, 2014 and received the same feeding regime described for experienced lambs. During summer of 2013, and prior to joining the experienced group of lambs, Control lambs consumed high-quality feedstuffs in pens from May to July 2013 (i.e., alfalfa pellets, barley grain, and beet pulp) and from August to October 2013 they



grazed irrigated high-quality pastures (tall fescue, sainfoin [*Onobrychis viciifolia*] and alfalfa).

*Experiment 1. Experience early in life and availability of alfalfa hay*

All animals were kept outdoors at the Green Canyon Ecology Center located in Logan, UT, USA. On May 6, 2014, all yearling sheep were individually penned outdoors, under a protective roof in individual, adjacent pens measuring  $1.5 \times 2.5$  m; alfalfa pellets were used as the basal diet until a day previous to harvested medusahead exposure (May 27). A period of adaptation to medusahead was not carried out because medusahead is an annual grass with a very short growth period. For instance, medusahead matured from the late vegetative to the late reproductive phenological stage in 17 days during June of 2013 (Chapter 4). Animals had free access to fresh water, salt and trace-mineralized salt blocks throughout the study. Research protocols were conducted according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval # 1551).

Experienced and inexperienced yearlings with an average body weight (BW) of  $58.7 \pm 5.7$  kg were blocked by BW and randomly assigned to one of four treatments: experience with medusahead + alfalfa hay ( $n = 8$ ), experience with medusahead without alfalfa hay ( $n = 7$ ), inexperience with medusahead + alfalfa hay ( $n = 7$ ), and inexperience with medusahead without alfalfa hay ( $n = 7$ ). One inexperienced animal died in the fall of 2013 and as a consequence each inexperienced group had 7 animals.

Alfalfa and endophyte-free tall fescue hays were passed through a hydraulic bale grinder (Gehl commercial forage grinder) and chopped into particles of 4-6 cm length.

A naturally established stand of medusahead growing in private land within the city limits of Paradise, Cache County, UT (41°34'03"N and 111°50'02"W) was harvested daily using a lawnmower (particle size 4-5-cm length) during the reproductive stage, transported to the Green Canyon Ecology Center, homogenized and fed to all yearlings on a daily basis.

Chopped alfalfa hay and chopped fresh medusahead were presented in two different feeders in each individual pen at 1000. The amount of chopped alfalfa hay was restricted to  $187.5 \pm 19.5$  g of dry matter (DM) per day (0.31% of initial BW) and it was consumed in less than 20 min. Thus, the empty feeders that contained alfalfa hay were removed daily from all pens at 1020. All animals had available chopped medusahead in *ad libitum* amounts between 1000 and 1300, and during this period events of medusahead intake (yes, no) were recorded at 5-minute intervals using the scan sampling technique (Altman, 1974). The proportion of events that each animal was observed consuming medusahead relative to the total number of events recorded during scan sampling was calculated daily. Medusahead refusals were removed at 1300 and intake was calculated by subtracting the amount of feed refused from the amount of feed offered. All animals were then offered chopped endophyte-free tall fescue hay in *ad libitum* amounts from 1300 to 1500. At 1500, refusals were collected and no other feed was offered until the next day. Tall fescue hay intake was estimated as described before. The experiment was conducted for 10 days, from May 28 to June 6, 2014.

Intake of salt blocks (NaCl [98.0-99.9%]; White Salt Block, North American Salt Company, Overland Park, Kansas, USA) and trace-mineralized salt blocks present in each pen (NaCl [93-98%], Zn [ $\geq 3500$  ppm], Mn [ $\geq 2800$  ppm], Fe [1750 ppm], Cu [350-

450 ppm], I [70 ppm], Co [70 ppm]; Morton iOfixt T-M, Chicago, IL, USA) were determined by subtracting the initial weight from the current weight of the block on days 2, 4, 6, 8, and 10 of the experiment. Salt and trace-mineralized salt block weights were determined at the same time (from 1000 to 1100) during those days.

Samples of offered forages and salt blocks were dried in an air forced oven at 60°C for 48 h to report intake values on DM basis. Forage samples were then ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) with a 1-mm screen for chemical analyses.

The biomass in the medusahead stand was estimated by harvesting the biomass at ground level in 6 squares (0.098 m<sup>2</sup>)/ha that were randomly dropped in the medusahead stand on the 7th day of the experiment. Samples were taken to the laboratory, manually sorted into medusahead, other annual grasses, bunch grasses, and forbs. The different functional groups were dried as described before and weighted to express their proportion in the plant community on a DM basis.

Yearlings were weighed at the end of the experiment and average daily BW gain and gain-to-feed ratio (kg of BW gain:kg of DM intake) were calculated.

## *Experiment 2. Effect of experience on grazing*

### *Study area*

All yearlings from Experiment 1 were transported to privately-owned land in Mantua, UT, USA (41° 29'51" N and 111°56'32" W). The ecological site is a Mountain Stony Loam, where the plant community is Mountain Big Sagebrush with introduced non-native species (Wadman, 2012); the study area had a moderate level of medusahead

infestation. Five blocks (0.18 ha/block), each divided into two plots (0.09 ha/plot) were marked on this area. Each plot was delimited using electric fence and a pen of  $\sim 3.0 \text{ m}^2$  was assembled outside each plot for overnight enclosure of the animals. Three control plots (no grazing) were located between the grazed blocks.

### *Experimental Protocol*

All yearling sheep had experience with ingesting medusahead after Experiment 1. However, just one group had experience early in life with grazing medusahead-infested rangeland. Sheep were randomly clustered into 10 groups of 3 yearlings each with the constraint that animals with and without experience early in life with grazing medusahead were kept in the same group ( $n = 5$  experience early in life with grazing medusahead;  $n = 5$  experience gained during Experiment 1). One group with experience gained during Experiment 1 had 2 yearlings, as one animal was lost in the fall of 2013. Groups were randomly assigned to the plots within each block with the constraint that an experienced and an inexperienced group grazed within each block (i.e., one group per plot). The experiment was conducted for 10 days (June 10 to 19) during the reproductive stage of medusahead. Animals had free access to water, salt and trace-mineralized salt blocks throughout the experiment.

Animals grazed from 0830 to 1700 and they were then penned overnight. The behavioral activity of yearlings was recorded at 5-min intervals from 0830 to 1100 and from 1600 to 1700 using the scan sampling technique (Altman, 1974). Foraging activities involved those events when animals were observed grazing medusahead, annual grasses other than medusahead, bunch grasses, and forbs in the plant community; other activities

were: walking, resting, ruminating, and drinking. The proportion of grazing events on medusahead and other functional groups in the community relative to the total number of scans recorded was determined daily. Animals were weighed at the end of the grazing trial to calculate the average daily BW change.

#### *Evaluation of the plant community*

Plant biomass production was estimated using a rising plate meter (Michell, 1982).

Twenty-five readings of plant height were recorded along a zig-zag transect per plot pre- and post-grazing using the rising plate meter (plate area = 0.0985 m<sup>2</sup>). Plant DM in each plot for calibration curves was assessed pre- and post-grazing by taking 27 random readings of the rising plate meter (Michell, 1982) during each period along with DM production values.

Soil cover was visually estimated in 25 squares (0.0985 m<sup>2</sup>/square), randomly distributed within a zig-zag transect per plot. The considered features of soil cover were: plants, stones ( $\geq 5$  mm), thatch (medusahead litter), litter (from plant material other than medusahead), and bare soil. Plant cover was also differentiated between standing and trampled plants. The relative fresh biomass abundance of medusahead and different plant functional groups (forbs, bunch grasses, and annual grasses other than medusahead) were visually estimated during soil cover estimations pre- and post-grazing. The relative frequency of medusahead and functional groups was estimated per plot (relative frequency = number of squares with medusahead or functional groups/total of squares x 100).

Four squares (0.0985 m<sup>2</sup>/square) within the aforementioned transect were randomly selected and harvested at the ground level. Plant material was taken to the

laboratory, manually sorted into medusahead and plant functional groups. A composite of medusahead and functional groups was formed with samples from 3 plots. Samples were dried in a forced air oven at 60°C until constant weight and ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) with a 1-mm screen for chemical analyses.

### *Medusahead defoliation*

Ten squares (0.009 m<sup>2</sup>) with medusahead tillers were marked on the soil of each plot; the squares were made with flagging tape, which was anchored to the soil with nails at each square corner; this was a modification for marking bunch grasses as described by O'Reagain and Grau (1995). A numbered flag was inserted into the soil about 40 cm from each square to target the area and reduce possible cueing effects on grazing behavior. Medusahead cover into each square was ranked from 1 to 5 according to the proportion of soil occupied by medusahead tillers: 1 = 10% cover (0.0009 m<sup>2</sup>), 2 = 30% cover (0.0027 m<sup>2</sup>), 3 = 50% cover (0.0045 m<sup>2</sup>), 4 = 70% cover (0.0063 m<sup>2</sup>), and 5 = 90% cover (0.0081 m<sup>2</sup>). Medusahead tillers within each square had similar height and the length of the tallest leaf in the tillers was measured. The estimated area of tillers multiplied by the height of the tallest leaf was used to estimate the initial volume of the biomass contained into the marked square immediately before grazing. On days 5 and 10 and after scan sampling, the proportion of defoliated tillers was visually estimated (defoliated area) and multiplied by the height of the tallest leaf remaining on the tillers to estimate the volume of biomass after grazing. The proportion of medusahead volume removed during grazing was calculated as: (initial volume – volume after grazing) / initial v

### *Chemical analyses*

Ground samples from Experiment 1 (medusahead collected on days 1 (May 28), 4 (May 31), 7 (June 3), and 10 (June 6), and alfalfa and tall fescue hays) and Experiment 2 (3 spatial replicates of medusahead, other annual grasses, bunch grasses, and forbs before and after grazing) were analyzed for crude protein (CP), neutral (NDF) and acid (ADF) detergent fiber, ash and acid insoluble ash (AIA). CP was calculated by measuring nitrogen content (Wiles *et al.*, 1998) and then by multiplying this concentration by 6.25. NDF and ADF contents were measured according to Van Soest *et al.* (1991), and ADF determination was sequential of NDF. Ash content was obtained by burning samples at 550°C for 6 h (Allen, 1989). Acid insoluble ash is an estimate of silica content (i.e., > 90% of AIA is silica [Charca *et al.*, 2007]) and it was determined by the method of 2N HCl (Van Keulen and Young, 1977).

### *Statistical analyses*

Analyses were computed using SAS (SAS Inst., Inc. Cary, NC; Version 9.1 for Windows). The covariance matrix structure used was the one that yielded the lowest Akaike information criterion (i.e., first-order autoregressive). The model diagnostics included testing for a normal distribution and homoscedasticity of data, and transformed when needed according to the Box-Cox method; non-transformed means  $\pm$  standard error of the mean (SEM) are reported. Means were analyzed using Tukey's multiple comparison tests when  $F$ -ratios were significant ( $P < 0.05$ ). A tendency was considered when  $0.05 < P < 0.15$ . One outlier (an inexperienced animal that did not receive alfalfa hay during Experiment 1) was removed from all analyses.

Feed intake, proportion of events recorded for eating medusahead during scan-sampling, average daily BW gain, and gain-to-feed ratio in Experiment 1 were analyzed as a mixed model in a 2x2 factorial design with early experience (yes, no) and availability of alfalfa hay (yes, no) as the main factors with sheep (random effect) nested within treatment group (experience and availability of alfalfa as fixed effects), and day (feed intake and scan-sampling data) or hour (scan sampling data) as the repeated measures.

The proportion of events for grazing medusahead, annual grasses, bunch grasses, and forbs, and percentage of medusahead volume removed during grazing in Experiment 2 were analyzed as a mixed-model effects with block as a random effect and experience and day as fixed effects. Average daily BW gain per group was analyzed using a one-way analysis of covariance with experience as a fixed factor and the average initial BW per group as a covariate.

The content of CP, NDF, ADF, ash, and AIA was compared using a two-way analysis of variance with functional group, time of sampling (pre- and post-grazing) and their interaction as the main factors. The chemical components of medusahead samples in Experiments 1 (Paradise) and 2 (Mantua) were compared using a one-way analysis of variance with sampling site as the main factor.



## Results

### *Experiment 1. Experience early in life and availability of alfalfa hay*

#### *Biomass production in the medusahead stand*

Biomass production in the medusahead stand was  $1535 \pm 358$  kg DM/ha. The composition of this stand (on a DM basis) was:  $78.4 \pm 8.9\%$  medusahead,  $19.4 \pm 8.6\%$  forbs,  $2.0 \pm 0.9\%$  bunch grasses and  $0.2 \pm 0.4\%$  annual grasses.

#### *Chemical composition of the forages*

Alfalfa hay showed the greatest content of CP followed by tall fescue hay (Table 3-1). ADF concentration was the greatest for alfalfa hay. Medusahead and tall fescue hay had the greatest concentrations of NDF, and medusahead showed the greatest contents of ash and AIA (Table 3-1). Concentrations of CP in medusahead decreased, whereas content of NDF, ADF, and ash increased towards the end of the experiment. Concentration of AIA in medusahead remained fairly constant throughout the study (Table 3-1).

#### *Intake of medusahead*

No difference in medusahead intake was detected between groups of yearlings with or without early experience with grazing medusahead ( $P = 0.4823$ ); average daily DM intake by experienced and inexperienced sheep was  $41.8 \pm 7.0$  g and  $37.6 \pm 8.9$  g, respectively. Availability of alfalfa did not favor intake of medusahead ( $P = 0.4486$ ); average daily DM intake by sheep with and without alfalfa hay was  $38.2 \pm 8.1$  g and  $37.0 \pm 5.8$  g, respectively. Likewise, alfalfa availability did not influence intake of medusahead by either the experienced or inexperienced group of animals (experience x

alfalfa availability effect;  $P = 0.2452$ ); average daily DM intakes were: experience with medusahead + alfalfa hay,  $46.1 \pm 8.2$  g; experience with medusahead without alfalfa hay,  $33.5 \pm 5.6$  g; inexperience with medusahead + alfalfa hay,  $34.6 \pm 10.5$  g; inexperience with medusahead without alfalfa hay,  $41.0 \pm 7.6$  g. Average intake data across days by groups of experienced and inexperienced sheep with or without access to alfalfa hay are presented in Figure 3-1A.

Sheep consumed different amounts of medusahead across days ( $P < 0.0001$ ; Figure 3-1B); they ate similar amounts of medusahead on days 1, 9 and 10 ( $P \geq 0.2518$ ), but they ingested more medusahead on day 1 than during days 2 through 8 ( $P \leq 0.0055$ ). Sheep also ate more medusahead on day 10 than on days 2, 3, 4, and 8 ( $P \leq 0.0184$ ) and tended to eat more medusahead on day 10 than on days 6 and 7 ( $P \leq 0.0973$ ). Thus, yearling sheep showed a cyclic pattern of medusahead intake, with peaks of greater intake during days 1 and 10.

Experienced and inexperienced sheep showed different intakes of medusahead across days (experience x day;  $P = 0.036$ ). Inexperienced sheep ate similar amounts of medusahead on days 1, 9 and 10 ( $P \geq 0.6367$ ; Figure 3-1C); however, intake of medusahead during day 1 was greater than intake values from days 2 through 8 ( $P \leq 0.0286$ ; Figure 3-1C). Furthermore, inexperienced sheep ate more medusahead on day 1 than experienced sheep during days 2, 3, 4, and 8 ( $P \leq 0.0258$ ; Figure 3-1C), and they tended to eat more medusahead during day 7 ( $P = 0.0697$ ). Thus, inexperienced sheep had a well-defined intake peak during the first day of medusahead exposure, followed by lower intake values during 7 days and a subsequent increment on days 9 and 10. Daily intake of medusahead by experienced animals was more even than for inexperienced

animals, as medusahead consumption by the experienced group did not differ across days ( $P > 0.05$ ; Figure 3-1C). Moreover, intake of medusahead by experienced animals on days 1, 5, 6, 7, 9, and 10 was not different from the peak value displayed by inexperienced animals during day 1 ( $P \geq 0.1860$ ) (Figure 3-1C).

Consistent with intake data, scan sampling did not reveal differences in medusahead use among treatment groups ( $P > 0.05$ ). Sheep showed different proportion of events eating medusahead according to day, hour, and hour by day ( $P < 0.0001$ ). A greater proportion of ingestive events on medusahead were recorded for day 10 ( $0.083 \pm 0.026$ ) than for days 2 ( $0.036 \pm 0.012$ ;  $P = 0.0087$ ), 3, 4, and 7 ( $< 0.021 \pm 0.000$ ;  $P < 0.0001$ ). The proportion of events recorded for eating medusahead during the first hour of feeding was 2X that observed during the second and third hours of feeding ( $0.068 \pm 0.022$  vs.  $0.029 \pm 0.012$  and  $0.029 \pm 0.012$ , respectively;  $P < 0.0001$ ).

#### *Intake of tall fescue hay*

Intake of tall fescue increased every day until day 5 ( $P < 0.0001$ ) as shown on Figure 3-2. Intake of tall fescue hay was not affected by experience, availability of alfalfa hay, or the interaction between these factors ( $P > 0.05$ ).

#### *Intake of salt blocks*

Sheep ate different amounts of salt across days ( $P = 0.0004$ ; Figure 3-3). Sheep ate more salt on day 10 than on day 8 ( $P = 0.0235$ ), and tended to eat more salt on day 6 than on days 8 ( $P = 0.0593$ ) and 4 ( $P = 0.1494$ ).

Averaged across days, experienced animals tended to eat more salt than inexperienced animals ( $11.8 \pm 3.9$  vs.  $5.4 \pm 2.0$  g, respectively,  $P = 0.0893$ ), and

experienced sheep tended to eat more salt than inexperienced sheep when both groups did not receive alfalfa ( $12.8 \pm 5.1$  vs.  $2.5 \pm 1.0$  g, respectively;  $P = 0.1287$ ). Intake of salt was not influenced by availability of alfalfa, experience x day, availability of alfalfa x day, or by experience x availability of alfalfa x day ( $P > 0.05$ ).

Sheep reduced intake of trace-mineralized salt across days ( $P < 0.0001$ ; Figure 3-3); they ate more trace-mineralized salt on day 2 than on days 4, 8, and 10 ( $P \leq 0.0017$ ), with an increment in intake on day 6 that was greater than intakes on days 8 and 10 ( $P \leq 0.0095$ ). Animals without alfalfa hay tended to eat more trace-mineralized salt blocks every 2 days than animals that received this forage ( $12.2 \pm 4.0$  vs.  $6.9 \pm 2.2$  g, respectively;  $P = 0.0859$ ). Experience and interactions between factors did not affect the intake of trace-mineralized salt ( $P > 0.05$ ).

#### *Average daily body weight gain and gain-to-feed-ratio*

Average daily BW gain tended to be greater for experienced than for inexperienced animals ( $306.2 \pm 44.6$  vs.  $191.8 \pm 27.2$  g/day, respectively;  $P = 0.0695$ ). Experienced animals showed a greater gain-to-feed ratio than inexperienced animals ( $0.29 \pm 0.05$  vs.  $0.20 \pm 0.01$  kg of BW gain/kg DM intake;  $P = 0.035$ ). Availability of alfalfa hay and its interaction with experience had no effect on average daily weight gain or gain-to-feed-ratio ( $P > 0.05$ ).

#### *Experiment 2. Effect of experience on grazing*

##### *Chemical composition of plants*

The chemical composition of the plant community where yearlings grazed medusahead-infested rangeland during Experiment 2 is shown in Table 3-2. Before grazing, plants had

greater content of CP ( $P < 0.0001$ ) and ash ( $P = 0.0016$ ) than plants sampled after grazing. Less mature plants had lower content of NDF ( $P = 0.0005$ ) and ADF ( $P < 0.0001$ ), and tended to have lower AIA ( $P = 0.1073$ ) than more mature plants.

Different functional groups had different content of CP, NDF, ADF, ash, and AIA ( $P < 0.0001$ ). Forbs and medusahead had the greatest content of CP and bunch grasses had the lowest content of CP ( $P \leq 0.0006$ ). Bunch grasses had the greatest content of NDF and ADF, and forbs showed the lowest concentration of these plant cell wall fractions ( $P \leq 0.0031$ ). Medusahead had the greatest content of ash and AIA; forbs had an intermediate content of ash but the lowest content of AIA, whereas annual and bunch grasses had the lowest content of ash, and intermediate content of AIA ( $P \leq 0.0346$ ).

There was no interaction between type of plant and sampling date for CP ( $P = 0.7529$ ), ash ( $P = 0.1212$ ), and AIA ( $P = 0.2848$ ) content. The NDF and ADF contents were different according to the type of plant and period of sampling ( $P = 0.0073$  and  $P = 0.0002$ , respectively) (Table 3-2).

Comparing the chemical composition of medusahead according to origin (Paradise and Mantua for Experiments 1 [Table 3-1] and 2 [Table 3-2], respectively), medusahead collected in Paradise had greater ash and AIA content than medusahead collected in Mantua ( $P = 0.0041$  and  $P = 0.0008$ , respectively). The NDF content tended to be greater in medusahead collected in Mantua than from that collected in Paradise ( $P = 0.1257$ ). Medusahead samples from different origin were not different in CP ( $P = 0.6366$ ) or ADF ( $P = 0.7250$ ) concentration.

### *Use of the plant community*

Sheep showed different proportion of grazing events during different days of the experiment ( $P < 0.0001$ ; Figure 3-4). Sheep increased the proportion of grazing events on day 3 ( $P \leq 0.0093$ ) which remained constant until day 6 ( $P > 0.05$ ). Grazing events increased again on day 7 relative to the previous day ( $P = 0.0285$ ) and they gradually declined, reaching the lowest value on day 10 ( $P = 0.0124$ ). Early experience with grazing medusahead-infested rangeland did not have an effect on the proportion of total grazing events recorded ( $P > 0.05$ ).

The proportion of events recorded for eating forbs decreased across days ( $P = 0.0033$ ); values were greater for day 3 than for days 9 and 10 ( $P \leq 0.0099$ ; Figure 3-4). A contrasting pattern was observed for scans recorded for ingestion of bunch grasses ( $P = 0.0001$ ); a lower proportion of ingestive events were recorded for days 1 and 2 than for days 7 and 9 ( $P < 0.05$ ; Figure 3-4). Early experience with grazing medusahead-infested rangeland or its interaction with day did not have an effect on the proportion of events recorded for grazing forbs and bunch grasses ( $P > 0.05$ ).

The proportion of events recorded for grazing annual grasses was not affected by early experience with grazing medusahead-infested rangeland, day, or their interaction ( $P > 0.05$ ; Figure 3-4.) The use of medusahead by sheep was almost nil; the proportion of grazing events on medusahead was  $0.0044 \pm 0.000$ .

### *Plant community*

Biomass availability before and after grazing is reported in Table 3-3. The average biomass removed in each grazed plot was  $104 \pm 22$  kg DM. During the same period, the biomass available in the ungrazed plots increased to  $172 \pm 76$  kg DM. Plants covered

more than the 50% of the soil and 26.5% of the plant cover was trampled after grazing (Table 3-3). The percentages of soil cover by rocks, litter and medusahead thatch are shown in the Table 3-3. Forbs were the most abundant resource in the plots, followed by bunch grasses, whilst medusahead and annual grasses showed a similar relative abundance before grazing (Table 3-3). After grazing, medusahead abundance increased whereas other annual grasses declined in their relative abundance (Table 3-3). Medusahead increased its relative frequency into each of the plots assessed (grazed and ungrazed) (Table 3-3). The relative frequency of annual grasses was reduced after grazing, but this variable increased in ungrazed plots (Table 3-3). Forbs and bunch grasses maintained their relative frequency after grazing (Table 3-3).

#### *Medusahead defoliation*

Animals with experience early in life at grazing medusahead tended to defoliate more volume of medusahead tillers than the inexperienced animals ( $P = 0.0995$ ), as the average proportion of defoliated volume every 5 days was  $0.022 \pm 0.011$  for experienced animals and  $0.006 \pm 0.000$  for inexperienced animals. Day or its interaction with experience early in life at grazing medusahead did not have an effect on the proportion of defoliated volume of medusahead tillers ( $P > 0.05$ ).

#### *Average daily body weight change*

Yearlings lost weight during the grazing trial, but there were no differences in the daily lost between experienced and inexperienced groups ( $P = 0.7488$ ),  $353.9 \pm 68.2$  g/day and  $338.5 \pm 46.7$  g/day, respectively. The average body weight of all animals after grazing was  $57.0 \text{ kg} \pm 1.0 \text{ kg}$ .

## Discussion

### *Cyclic pattern of medusahead intake*

Intake of medusahead by yearling sheep was low and cyclic, which is consistent with previous research conducted in lambs (Hamilton *et al.*, 2015; Villalba and Burritt, 2015). Feed intake across days by herbivores becomes cyclic (i.e., gradual increases followed by declines in food ingestion) when animals eat a certain food and then they experience negative post-ingestive consequences due to an excess of nutrients released into the gastrointestinal tract from rapidly fermentable foods, due to nutrient deficiencies or due to toxins (Provenza, 1996). Medusahead is not toxic (Hamilton *et al.*, 2015) or rich in nutrients but high in the concentration of the anti-nutritional component silica, as shown in this study by the high content of AIA recorded for this weed. Silica is involved in the reduction of preference for grasses by herbivores and this effect is more pronounced as its content in plant tissues increases (Massey *et al.*, 2009). The effect of this anti-nutritional factor may contribute to the cyclic pattern of medusahead intake by sheep found during experiments conducted in confinement (see Chapter 4).

The interaction between the sensorial characteristics of feeds (sensory-specific responses) and their post-ingestive effects (nutrient-specific responses) affect preference, being difficult to isolate the independent effects of either (Provenza and Villalba, 2006). Animals decrease feed intake when they only receive chemo-sensorial stimulation or chemo-sensorial stimulation matched with intragastric infusions of non-nutritive solutions (Rolls, 1986). This response may be due to the lack of positive post-ingestive feedback that typically stimulates feeding (Provenza, 1996). The silicified epidermis in grasses represents a physical barrier (Mayland and Shewmaker, 2001) that decreases the



breakup of forages and release of nutrients (Bae *et al.*, 1997; Hunt *et al.*, 2008), which decreases the rate of fermentation as observed for medusahead (i.e., Chapter 4). Thus, the slow availability of nutrients from medusahead may produce weak positive post-ingestive feedback signals that do not encourage further consumption or preference for this weed. During Experiment 1, yearlings ate approximately half of their daily medusahead intake during the first hour of feeding and subsequently medusahead intake decreased. Ruminants may detect the low nutrient release from a low-quality food in their gastrointestinal tract within minutes after the initiation of the ingestive event as post-ingestive feedback from nutrients begins within minutes after food ingestion (Provenza, 1996).

Early experience with medusahead played a role on the pattern of medusahead intake across days. Peaks and nadirs of medusahead intake were more evident for inexperienced animals, whereas intake was more even for experienced yearlings. The slow breakup and fermentation of medusahead could increase fill effects in the rumen and reduce passage rate through the reticulorumen (Chapter 4). Considering that positive experiences early in life can have life-long influences on herbivores by causing neurological, morphological, and physiological changes (Provenza and Balph, 1990), experienced yearling could have had a greater number of rumination events - the major force in reducing particle size (Van Soest, 1994) - thus reducing fill effects and increasing the passage rate of medusahead. This effect was likely enough to change the feeding pattern but not high enough to promote greater differences in daily feed intake.

The availability of alfalfa hay did not change the nutritional context to favor an increment in medusahead intake by sheep. It is worth mentioning that alfalfa is a legume

of high protein content (National Research Council, 2007) and Hamilton *et al.* (2015) reported that lambs tended to eat more medusahead when they were supplemented with a high-energy concentrate. Nevertheless, the presence of alternative high-quality foods do not typically lead to clear increments in medusahead intake by sheep (Hamilton *et al.*, 2015; Villalba and Burritt, 2015) likely due to the aforementioned digestibility constraints imposed by silica which are reported in greater detail in Chapter 4.

In addition to cyclic patterns of intake, there is a great deal of variation in medusahead intake among individuals. This variability was also observed during this study by yearlings with and without experience, and in lambs with different types of supplement (energy and protein concentrates; Hamilton *et al.*, 2015), or energy concentrates containing different feed additives (Ca-propionate or yeast culture; Villalba and Burritt, 2015).

### *Salt intake*

Sheep display an innate appetite for sodium (NRC, 2007) and sodium deficiencies are not typical in ruminants (Bell, 1972; Valk and Kogut, 1998). Hubbert *et al.* (1958) reported that it appeared desirable to maintain a certain ratio between the concentration of sodium and potassium for maximum cellulose digestion on *in vitro* trials, while Cl did not have an effect on digestibility. Additionally, fertilization of perennial ryegrass (*Lolium perenne*) with sodium increased DM digestibility, while it increased Na content, decreased K content, with no apparent change in CP and fiber concentrations in the plant's tissues (Chiy and Phillips, 1998).

Grasses are relatively rich in minerals, with low contents of sodium and high concentrations of potassium (Bell, 1972). Sodium content in plants varies seasonally and

many grazers, including sheep, may become sodium deficient (NRC, 2007). Experienced yearlings likely learned to consume more salt when they grazed with their mothers as lambs on a plant community with moderate to low Na content (Chapter 2). Thus, when experienced yearlings were exposed to medusahead and tall fescue hay, they likely increased their Na intake relative to Controls to balance the low Na:K ratio found in grasses like tall fescue (i.e., 24.5 g K and 0.12 g Na/kg DM; Swift *et al.*, 2007) and medusahead (i.e., 12.0 g K/kg DM; Bovey *et al.*, 1961).

Sheep fed alfalfa tended to eat less trace-mineralized salt blocks than sheep that did not receive this forage. Alfalfa hay supplied some of the yearlings' trace-mineral requirements (i.e., Zn; NRC, 2007), which likely led to a reduction in the use of blocks by supplemented animals.

### *Animal performance*

During Experiment 1, experienced animals tended to gain more weight and showed better gain-to-feed ratio than inexperienced animals. Consistent with these findings, lambs exposed to low-quality roughages early in life showed greater *in vivo* digestibility values when fed a different low-quality forage later in life than lambs without such early experience (Distel *et al.*, 1994). Likewise, beef cows exposed to a low-quality forage early in their lives had a better body condition score, body weight and reproductive parameters than inexperienced animals when fed the same low-quality forage later in life (Wiedmeier *et al.*, 2002). Early exposure by mammals to different feeds and locations may lead to changes in gene expression, which allow for a better adaptation to the animal's local feeding environment (Provenza, 2008).

Minerals also have an important role in body weight gains. Croom *et al.* (1982) and Rossi *et al.* (1998) reported that NaCl could, in some circumstances, increase feed efficiency in ruminants. Sodium forms part of diverse transport systems (i.e., Na/K pump) that are important in glucose and amino acid uptake by tissues, maintenance of body temperature, neuromuscular activation, and nerve function (NRC, 2007). Experienced yearlings tended to consume more salt than Controls, which could have contributed to the differences in BW and gain-to-feed ratio found between groups. Initial BW (58.7 kg) indicated that yearlings were close to their mature weight (i.e., 62.5 kg; NRC, 2007). Thus, the weight gain recorded in pens was likely due to fat accretion (NRC, 2007).

During Experiment 2, all animals lost weight and early experience with grazing medusahead did not influence this variable. The poor quality of the plant community compounded with the greater maintenance requirements for animals under grazing conditions (Owens *et al.*, 1993; Van Soest, 1994) may have contributed to this outcome, which likely overrode the positive effects of early experience on BW gains found during Experiment 1.

#### *Use of plant community on medusahead infested rangeland*

Animals become averse to foods eaten too frequently or in excess; the more of any particular food an animal eats, the greater the aversion to the food. The degree to which a food is avoided following a meal depends on such excessive exposure compounded with the presence of toxins in the food or with the occurrence of nutrient imbalances (Provenza, 1996). It is likely that yearlings became averse to medusahead due to the excessive exposure they received to this low-quality weed during Experiment 1. This

resulted in the almost nil use of medusahead observed during grazing in Experiment 2. Yearlings were observed grazing medusahead for only 0.44% of the events recorded during scan sampling, which contrasts with the value of 4.4% recorded when they grazed medusahead-infested rangeland with their mothers as lambs (Chapter 2). Nevertheless, despite the low proportion of grazing events recorded, experienced animals tended to defoliate more medusahead tillers than inexperienced animals.

On rangelands, availability of different sward components in the vegetation becomes important as it can limit the expression of the consumers' feeding preferences. The less available a component is, the less likely it will form part of the animals' diet. Herbivores broadly switch to the plant species that are most available as the abundance of their preferred species decreases, and this behavior allows them to maintain feed intake in accordance to their nutrient requirements (Dumont, 1997). At the beginning of the grazing experiment, the use of forbs, bunch grasses, and annual grasses by yearlings was proportional to their abundance; however, as days elapsed sheep changed the use of different plant functional groups with a gradual reduction in forb defoliation – a preferred component of the vegetation that declined in abundance - and an increase in the use of grasses. It has been observed that grazing animals change their diet selection in an attempt to rebalance the ingestion of nutrients as a function of their changing requirements (Parsons *et al.*, 1994). In addition, the synchronization of nitrogen and carbohydrates for microbial metabolism is necessary for a better utilization of substrates (Gárces-Yépez *et al.*, 1997).

The low and cyclic intake of medusahead showed by yearling sheep and the growth of medusahead in patches can be used as the basis for prescribed grazing in

medusahead-infested rangeland. When the level of infestation is low (i.e., < 10% medusahead abundance), using a moderate animal density and animals which were not previously forced to consume medusahead (e.g., lambs with their mothers that graze medusahead to 7.5% of the grazing events recorded) may contribute to prevent the spread of the weed, as animals will consume medusahead in proportion to its abundance. Animals previously forced to graze medusahead, even if they consumed the weed early in life with their mothers, may display a much lower use of the weed as shown in this study (e.g., 0.44% of the total observed behavioral events or 0.61% of the grazing events recorded). On the other hand, for rangelands with much higher levels of infestation, animals may be managed to grazing cycles where they are concentrated in medusahead-infested patches for a short period of time (i.e., 1 day), moved to a diverse vegetation for 3-7 days, and then returned to medusahead patches for another short period of time. This rotation will make a more efficient use of the weed, as it will mimic the described cyclic pattern of medusahead intake by sheep exposed to *ad libitum* amounts of this weed. In addition, experienced animals in this context may use medusahead more evenly and display better BW gains than inexperienced animals. Nevertheless, it is worth mentioning that the proposed management approach should be avoided during the period of seed disarticulation, thus preventing the potential long-distance dispersal of medusahead seed via epizoochory.

## Conclusions

Early experiences with grazing medusahead or supplementation with alfalfa did not enhance medusahead intake later in life by sheep. Intake of medusahead was low and cyclic, likely due to the high concentrations of silica in the plant's tissues, with a more

even consumption of medusahead by the experienced group of animals. Early experience with medusahead favored weight gains and gain-to-feed ratio only when animals received medusahead in *ad libitum* amounts and then a basal diet of tall fescue hay. Alfalfa supplementation likely contributed with minerals that caused the reduction of trace-mineralized salt block intake. Experienced sheep also showed a greater intake of NaCl, which could be explained by their early experiences with grazing a plant community poor in Na. Finally, it is likely that forcing animals to ingest medusahead in pens attenuated the subsequent use of medusahead during grazing.

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**Table 3-1** Chemical composition (mean  $\pm$  SD) of the forages fed during Experiment 1.

Forage	Content (g/kg dry matter)				
	CP	NDF	ADF	Ash	AIA
Medusahead (May 28 to June 6 of 2014)					
Day 1, May 28	89.4 $\pm$ 4.6	613.5 $\pm$ 17.0	308.3 $\pm$ 12.9	167.9 $\pm$ 0.1	108.9 $\pm$ 0.1
Day 4, May 31	100.6 $\pm$ 2.7	616.5 $\pm$ 0.8	303.2 $\pm$ 5.0	154.6 $\pm$ 0.1	100.3 $\pm$ 0.2
Day 7, June 3	91.4 $\pm$ 2.3	589.2 $\pm$ 9.6	302.8 $\pm$ 5.6	170.4 $\pm$ 0.8	109.9 $\pm$ 0.7
Day 10, June 6	70.8 $\pm$ 0.7	641.7 $\pm$ 6.8	331.6 $\pm$ 8.8	179.5 $\pm$ 3.5	107.5 $\pm$ 0.2
Alfalfa hay	197.1 $\pm$ 1.0	464.8 $\pm$ 10.0	340.3 $\pm$ 8.0	93.1 $\pm$ 1.2	23.0 $\pm$ 0.4
Tall fescue hay	153.3 $\pm$ 0.2	607.1 $\pm$ 8.3	301.6 $\pm$ 3.4	104.1 $\pm$ 0.1	32.4 $\pm$ 0.4

CP = crude protein

NDF = neutral detergent fiber

ADF = acid detergent fiber

Ash = mineral fraction

AIA = acid insoluble ash (> 90% is silica; Charca *et al.*, 2007)

Chemical analyses of 1 replicate

**Table 3-2** Chemical composition (mean  $\pm$  SEM) of the plant community where sheep grazed during Experiment 2.

Plant group	Content (g/kg dry matter)				
	CP	NDF	ADF	Ash	AIA
Pre-grazing (June 9 of 2014)					
Medusahead	96.2 $\pm$ 6.8 <sup>ab</sup>	623.1 $\pm$ 2.5 <sup>ab</sup>	305.2 $\pm$ 4.7 <sup>ab</sup>	128.4 $\pm$ 6.1 <sup>a</sup>	82.0 $\pm$ 7.9 <sup>a</sup>
Annual grasses	84.5 $\pm$ 1.3 <sup>bc</sup>	575.8 $\pm$ 1.1 <sup>b</sup>	292.5 $\pm$ 3.7 <sup>a</sup>	76.6 $\pm$ 3.6 <sup>cd</sup>	36.6 $\pm$ 0.6 <sup>b</sup>
Bunch grasses	71.7 $\pm$ 5.3 <sup>c</sup>	687.4 $\pm$ 11.3 <sup>ac</sup>	312.3 $\pm$ 2.2 <sup>ab</sup>	77.0 $\pm$ 2.9 <sup>cd</sup>	38.7 $\pm$ 3.3 <sup>b</sup>
Forbs	102.2 $\pm$ 4.2 <sup>a</sup>	376.0 $\pm$ 9.9 <sup>d</sup>	258.1 $\pm$ 5.3 <sup>c</sup>	103.3 $\pm$ 2.3 <sup>b</sup>	5.4 $\pm$ 0.8 <sup>c</sup>
Post-grazing (June 20 of 2014)					
Medusahead	71.5 $\pm$ 3.7 <sup>ab</sup>	645.1 $\pm$ 4.7 <sup>a</sup>	323.8 $\pm$ 6.0 <sup>bd</sup>	101.4 $\pm$ 1.7 <sup>b</sup>	80.6 $\pm$ 7.9 <sup>a</sup>
Annual grasses	59.2 $\pm$ 1.1 <sup>bc</sup>	676.0 $\pm$ 24.2 <sup>ac</sup>	351.7 $\pm$ 13.2 <sup>dc</sup>	67.3 $\pm$ 6.2 <sup>d</sup>	38.3 $\pm$ 6.0 <sup>b</sup>
Bunch grasses	49.9 $\pm$ 4.6 <sup>c</sup>	737.4 $\pm$ 23.9 <sup>c</sup>	402.8 $\pm$ 12.5 <sup>e</sup>	68.9 $\pm$ 7.9 <sup>d</sup>	43.4 $\pm$ 2.8 <sup>b</sup>
Forbs	80.5 $\pm$ 4.8 <sup>a</sup>	373.7 $\pm$ 2.2 <sup>d</sup>	256.4 $\pm$ 1.3 <sup>c</sup>	98.2 $\pm$ 3.2 <sup>cb</sup>	7.2 $\pm$ 0.3 <sup>b</sup>

CP= crude protein

NDF= neutral detergent fiber

ADF= acid detergent fiber

Ash= mineral fraction

AIA= acid insoluble ash (> 90% is silica; Charca *et al.*, 2007)

Chemical analyses of 3 replicates

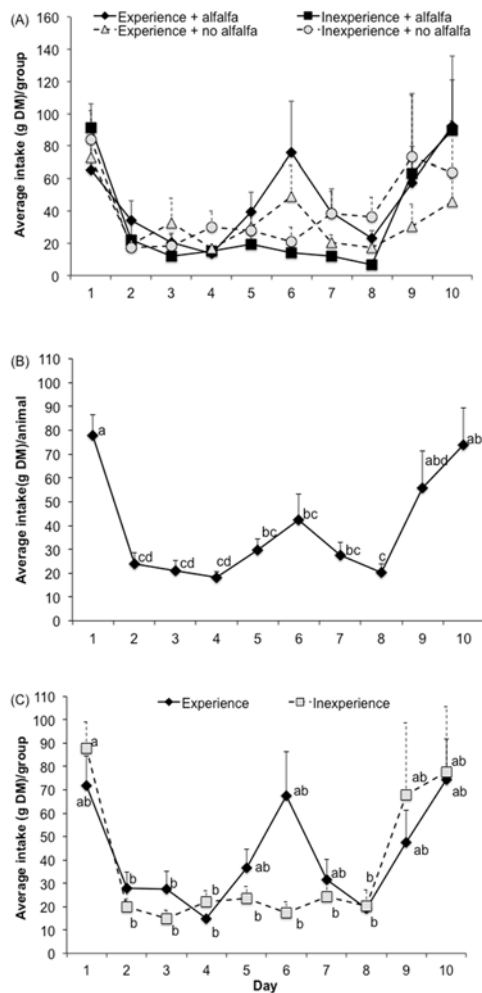
Annual grasses = this group included annual grasses other than medusahead

Values within a row with same superscripts did not have difference ( $P > 0.05$ )

**Table 3-3** Relative soil cover (mean  $\pm$  SEM), relative abundance and frequency of plant functional groups in the plots grazed by experienced and inexperienced animals, and in ungrazed plots (Control).

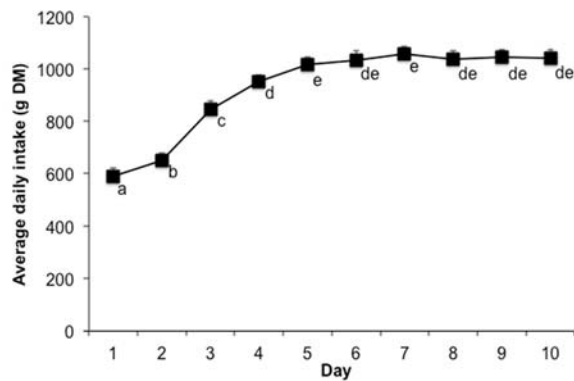
Item	Pre-grazing			Post-grazing		
	Experience	Inexperience	Control	Experience	Inexperience	Control
Biomass, kg of dry matter/plot (0.09 ha)	354 $\pm$ 22	348 $\pm$ 31	358 $\pm$ 20	226 $\pm$ 32	268 $\pm$ 14	531 $\pm$ 64
Relative percentage of soil cover						
Standing plants	53.8 $\pm$ 3.1	66.0 $\pm$ 4.6	67.2 $\pm$ 1.6	46.2 $\pm$ 3.2	56.6 $\pm$ 6.8	66.4 $\pm$ 7.6
Trampled plants	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	17.8 $\pm$ 4.4	19.3 $\pm$ 5.7	0.0 $\pm$ 0.0
Bare soil	25.9 $\pm$ 2.9	19.4 $\pm$ 1.9	8.8 $\pm$ 3.8	15.9 $\pm$ 3.1	14.4 $\pm$ 3.0	10.3 $\pm$ 4.3
Rocks	6.3 $\pm$ 1.6	5.3 $\pm$ 1.4	3.7 $\pm$ 2.1	4.6 $\pm$ 0.9	4.7 $\pm$ 1.9	3.3 $\pm$ 1.4
Litter	13.4 $\pm$ 1.4	9.1 $\pm$ 2.5	18.5 $\pm$ 5.1	15.4 $\pm$ 3.7	5.0 $\pm$ 3.1	18.8 $\pm$ 2.9
Thatch	0.6 $\pm$ 0.3	0.2 $\pm$ 0.1	1.9 $\pm$ 1.3	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	1.1 $\pm$ 0.9
Relative abundance (%) of medusahead and different plant functional groups						
Medusahead	7.6 $\pm$ 1.9	5.0 $\pm$ 0.8	7.7 $\pm$ 0.1	9.5 $\pm$ 1.6	10.1 $\pm$ 1.5	10.3 $\pm$ 0.3
Annual grasses	5.8 $\pm$ 1.7	6.7 $\pm$ 2.5	9.3 $\pm$ 6.6	2.6 $\pm$ 1.0	2.9 $\pm$ 1.4	11.5 $\pm$ 2.7
Bunch grasses	30.6 $\pm$ 3.2	29.6 $\pm$ 2.6	24.3 $\pm$ 1.6	37.5 $\pm$ 3.0	35.7 $\pm$ 3.8	21.8 $\pm$ 2.4
Forbs	56.0 $\pm$ 4.0	58.7 $\pm$ 2.9	58.7 $\pm$ 3.6	50.3 $\pm$ 4.1	51.2 $\pm$ 3.1	56.4 $\pm$ 1.4
Relative frequency (%) of different plant functional groups						
Medusahead	43.2 $\pm$ 4.6	39.2 $\pm$ 2.9	34.7 $\pm$ 5.3	48.8 $\pm$ 4.9	46.4 $\pm$ 3.2	44.0 $\pm$ 1.1
Annual grasses	28.0 $\pm$ 11.3	38.4 $\pm$ 3.5	48.0 $\pm$ 2.3	11.2 $\pm$ 4.9	34.4 $\pm$ 7.4	56.0 $\pm$ 6.1
Bunch grasses	97.6 $\pm$ 2.4	96.0 $\pm$ 1.2	82.7 $\pm$ 9.6	97.6 $\pm$ 1.6	98.4 $\pm$ 1.0	96.0 $\pm$ 0.0
Forbs	99.2 $\pm$ 0.8	100.0 $\pm$ 0.0	96.0 $\pm$ 2.3	98.4 $\pm$ 1.0	95.2 $\pm$ 2.3	98.7 $\pm$ 1.3

Annual grasses= Annual grasses other than medusahead



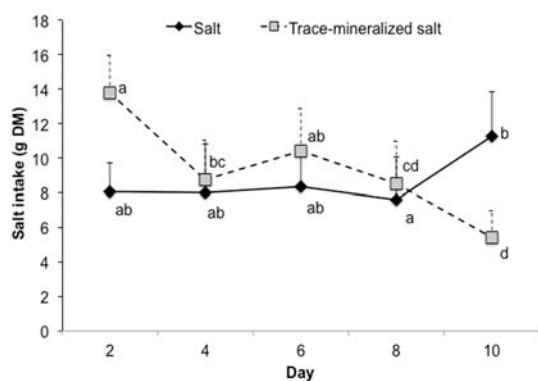
**Figure 3-1** Average daily dry matter (DM) intake of medusahead by four groups of yearling sheep during 3-h of exposure in pens (Experiment 1). (A) Average daily intake of medusahead by the four groups: with (Experience) or without (Inexperience) early experience at grazing medusahead-infested rangeland and with (alfalfa) or without (no alfalfa) alfalfa hay availability; (B) average daily intake of medusahead across the four groups, and (C) average daily intake of medusahead across the 2 levels of alfalfa availability (alfalfa, no alfalfa) by sheep with and without experience at grazing medusahead-infested rangeland. Values are means with SEM. Means with the same letter did not differ ( $P > 0.05$ ) between days (B) or between groups on different days (C).



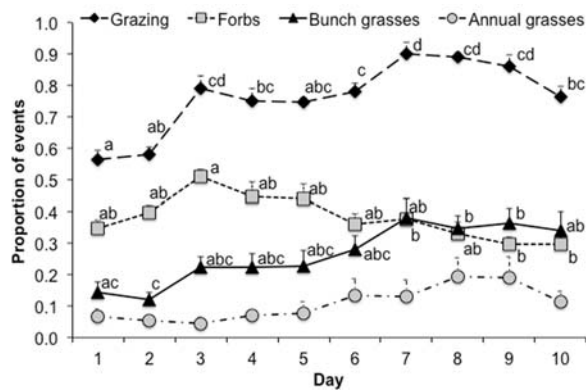


**Figure 3-2** Average daily dry matter (DM) intake of tall fescue hay during Experiment 1.

Tall fescue hay was the basal diet of sheep fed in pens. Values are means with SEM across four groups of animals: with or without early experience at grazing medusahead-infested rangeland and with or without alfalfa hay availability. Means with same letter did not differ ( $P > 0.05$ ).



**Figure 3-3** Intake of salt (NaCl) and trace-mineralized salt blocks (NaCl + microminerals [Zn, Mn, Fe, Cu, I, and Co]) by sheep measured every 2 days (Experiment 1). Values are means with SEM across four groups of animals: with or without early experience at grazing medusahead-infested rangeland and with or without alfalfa hay availability. For each salt block, means with the same letter did not differ ( $P > 0.05$ ).



**Figure 3-4** Proportion of total grazing events and grazing events on different functional groups by sheep grazing on medusahead-infested rangeland during 3.5 h of scan sampling (Experiment 2). Values are means with SEM across two groups of animals: with or without early experience at grazing medusahead-infested rangeland. Annual grasses group include annual grasses other than medusahead. For each event, means with the same letter did not differ ( $P > 0.05$ ).

## CHAPTER 4

UNDERSTANDING MEDUSAHEAD (*TAENIATHERUM CAPUT-MEDUSAE* SSP.  
*ASPERUM*) LOW INTAKE AND PALATABILITY THROUGH *IN VITRO*  
 DIGESTIBILITY AND FERMENTATION KINETICS<sup>1</sup>

**Abstract**

The low digestibility of medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) - attributed to high concentrations of amorphous silica - may negatively impact intake and preference by herbivores, making this weed a successful competitor in grazed plant communities. The goals of this study were to determine the influence of (1) stage of plant maturity (from late vegetative to beginning of senescence and thatch), (2) particle size (1, 5, 10, and 20 mm), and (3) high-quality feeds (alfalfa hay and a high-energy concentrate) on fermentation kinetics and apparent digestibility of medusahead relative to more palatable feeds (alfalfa and tall fescue hays and high-energy concentrates). Finally (4), the influence of soluble silica (SiO<sub>2</sub>) on the fermentation kinetics and apparent digestibility of alfalfa hay was determined. *In vitro* gas production was estimated at 2, 4, 6, 8, 12, 18, 24, 36, 48, and 72 h of incubation, and apparent dry matter and organic matter digestibility (OMD) of the substrates were assessed after incubation. Medusahead samples, except thatch, had greater ( $P < 0.05$ ) OMD (65-71%) than alfalfa hay (53%), similar to tall fescue hay (67%;  $P > 0.05$ ) and lower ( $P < 0.05$ ) than the high-energy concentrates assayed (77-79%). Medusahead thatch had the lowest digestibility values recorded (46%;  $P < 0.05$ ). Fermentation kinetics showed slow fermentation rates for

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medusahead relative to alfalfa ( $P < 0.05$ ), and a decline in fermentation rates with plant maturity ( $P < 0.05$ ). Fermentation rates of the substrates were reduced with particle sizes  $\geq 5$  mm ( $P < 0.05$ ), and apparent OMD for medusahead declined as particle size increased, a relationship not found for alfalfa or tall fescue hays ( $P < 0.05$ ). No associative effects on digestibility parameters were observed between medusahead and the high-quality feeds tested ( $P > 0.05$ ). The addition of SiO<sub>2</sub> did not have an effect ( $P > 0.05$ ) on OMD of alfalfa hay but it reduced gas production during the first hours of incubation ( $P < 0.05$ ). We conclude that fermentation kinetics of medusahead -instead of its final apparent digestibility- contributes to explain its low intake and palatability. A significant inhibitory effect of particle size on medusahead digestibility –likely mediated by the negative (i.e., mechanical, chemical) effects of silica on medusahead fermentation– also contributes to explain the low use of this weed by herbivores. Such inhibitory effect may also underlie the lack of associative effects observed during the study.

## 1. Introduction

Medusahead (*Taeniatherium caput-medusae* ssp. *asperum*) is a Eurasian grass that has been invading rangelands in the western United States since its introduction at the end of the 19th century (Young, 1992; Davies and Johnson, 2008). It has been estimated that until 2005 medusahead had infested about 1 million hectares in this region (Duncan and Clark, 2005), an invasion that entails a significant problem for the structure and function of natural ecosystems (Young et al., 1999; Masters and Sheley, 2001; Sheley and James, 2010).

Prescribed grazing is a successful and sustainable tool for the control of exotic invasive plant species (Frost and Launchbaugh, 2003; Vavra et al., 2007). However, for the particular case of medusahead, intake by livestock is very low and variable (Lusk et al., 1961; Young, 1992), which has been recently confirmed in controlled cut-and-carry feeding trials with sheep (Hamilton et al., 2015; Villalba and Burritt, 2015).

The low palatability of medusahead could be explained by its low nutritional value. However, the protein and fiber content of this weed are similar to values reported for nutritive grasses that are highly palatable (Bovey et al., 1961; Shawrang and Nikkhah, 2005; NRC, 2007; Villalba and Burritt, 2015). In contrast, the concentration of silica in medusahead is particularly high in relation to other forages with values ranging from 12 to 19% on a dry matter (DM) basis, depending on plant phenology and environmental factors (Bovey et al., 1961; Swenson et al., 1964; Mutch and Philpot, 1970). This anti-nutritional component is mainly present in the form of amorphous silica ( $[\text{SiO}_x(\text{OH})_{4-2x}]_n$ ) in the cell walls of the epidermis of the leaves, stems, awns, and glumes of medusahead (Swenson et al., 1964). The epidermal silica in plants is a varnish that limits the degradation of unsilicified organic constituents underneath (Van Soest and Jones, 1968; Mayland and Shewmaker, 2001; Van Soest, 2006). In addition, the silicified waxy cuticular layer and cell wall may work as a hard physical barrier resistant to mechanical breakdown (chewing and rumination), reducing the breakdown rate of feed particles in the rumen (Van Soest, 1994; Bae et al., 1997; Hunt et al., 2008) which could further reduce food intake and palatability (Allen, 1996).

Silica is also found as soluble silica (orthosilicic acid,  $\text{Si}(\text{OH})_4$ ) in different plant tissues (Currie and Perry, 2007). When present in the liquid phase, soluble silica reduces

forage digestibility (Smith and Nelson, 1975) by inhibition of digestive enzymes such as a cellulase (Shimojo and Goto, 1989), and likely by its interaction with cations (Belton et al., 2012), basic amino acids, peptides, and proteins (Currie and Perry, 2007; Neethirajan et al., 2009) in the digestive tract. Reductions in digestibility and in preference for grasses high in silica content have been reported for voles (Massey and Hartley, 2006; Massey et al., 2008), rabbits (Cotterill et al., 2007), and sheep (Massey et al., 2009).

Chemical and biological treatments are being used to increase digestibility of forages high in silica like rice straw (i.e., 130 g silica/kg; Van Soest, 2006). Nevertheless, these treatments are unpractical for standing grasses in rangelands like medusahead. Supplementation with readily degradable fiber sources (i.e., beet pulp) (Caton and Dhuyvetter, 1997; Garcés-Yépez et al., 1997), forages high in protein like alfalfa (Van Soest, 1994), or glucogenic precursors like Ca propionate (Villalba and Burritt, 2015) have been suggested as options to favor fiber digestion and/or intake of low-quality forages like medusahead.

We hypothesized that plant phenology, silica content, nutrients and particle size influence the ruminal degradability of medusahead which in turn impact food intake and palatability. Thus, the goals of this study were to measure the apparent digestibility and fermentation kinetics of medusahead relative to more palatable forages at different phenological stages and different particle sizes, and when associated with supplementary nutrients. Finally, the influence of soluble silica on the fermentation kinetics and apparent digestibility of alfalfa hay was determined.

## 2. Materials and methods

### 2.1 Substrates

#### 2.1.1 Experiment 1: Digestibility of medusahead at different phenological stages relative to that of other forages and concentrates

Medusahead samples were collected in June 2013, from medusahead-infested private land located in Mantua, UT, USA (41° 29'51" N and 111°56'32" W), where natural vegetation is classified as Mountain Big Sagebrush (Wadman, 2012).

Medusahead was harvested at the ground level at three spots in 1.35-ha area, taken to the lab and homogenized to create representative samples from different stages of maturity: a) late vegetative (June 1; M1), b) mid reproductive (June 11; M2), c) late reproductive (June 17; M3), d) senescent (June 27; M4), and e) thatch (dead matter from previous years; T) (June 27). Additionally, a proportion of M3 sample was manually sorted into inflorescence (M3I), which included the spike and 5 mm of peduncle, and leaves + stems (M3L). M3I and M3L represented 49% and 51% of the whole plant on DM basis, respectively.

Two palatable forages, alfalfa (*Medicago sativa*) hay (AH), and endophyte-free tall fescue (*Festuca arundinacea*) hay (FH), and two similar energy concentrates were used as controls. The high-energy concentrates were: (1) beet pulp, barley grain, and Ca-propionate (Sigma-Aldrich, St. Louis, MO, USA), mixed in a 66:30:4 as-fed ratio (CaP), and (2) beet pulp, barley grain, and yeast culture (*Saccharomyces cerevisiae* fermentation product; Diamond V XP, Diamond V Mills, Cedar Rapids, IA, USA), mixed in a 65:30:5 as-fed ratio (YC). These feedstuffs were chosen because they were used in previous pen and grazing studies as a basal diet (FH) or supplement (AH, high-energy concentrates) to



explore the influence of nutrients on medusahead use by sheep (Hamilton et al., 2015; Villalba and Burritt, 2015; Chapters 2 and 3).

All samples were dried in a forced air oven at 60°C for 48 h and ground to pass a 1-mm screen, according to the common particle size for *in vitro* gas production measurement (Menke and Steingass, 1989; Theodorou et al., 1994).

### *2.1.2 Experiment 2: Digestibility of substrates at different particle sizes*

AH, FH, and M2 samples were cut manually to 5, 10, and 20 mm of length, and used, including the 1-mm particle size to determine forage apparent digestibility and fermentation kinetics. M2 was selected for its intermediate chemical quality (Villalba and Burritt, 2015) between tissues collected at the vegetative and reproductive phenological stages.

### *2.1.3 Experiment 3: Digestibility of medusahead mixtures – Associative effects*

Medusahead (M2) was mixed with AH or CaP to test for associative effects. Mixtures of M2:AH and M2:CaP (ground to 1-mm particle size) were prepared to measure digestibility and fermentation kinetics: medusahead (M2) was mixed with AH or CaP in proportions of 50:50, 70:30, and 90:10, respectively. Alfalfa hay and energy concentrates (beet pulp and barley as main ingredients) have been used in previous studies as supplements to enhance use of medusahead by sheep (Hamilton et al., 2015; Villalba and Burritt, 2015; Chapters 2 and 3).

#### *2.1.4 Experiment 4. Effect of soluble silica on alfalfa hay digestibility*

Soluble silica (SiO<sub>2</sub>, approx. 80% between 1-5 µm [0.5-10 µm], Sigma-Aldrich, San Louis, MO, USA) was added to AH (ground to 1-mm particle size) in proportions of 2.5 and 5.0% of the mix.

### *2.2 In vitro gas production*

#### *2.2.1 Inoculum*

Two ruminally-cannulated commercial yearling cross-bred wethers were handled according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval # 1551). Rumen liquid was collected 4 h post-feeding from both animals, which were fed daily at 0700 *ad libitum* amounts of endophyte-free tall fescue grass hay (~2.5 kg/day). Rumen liquid was vacuumed from each sheep and transferred to 2 pre-warmed (39°C) thermal flasks of 500 ml each. Samples were immediately transported to the laboratory, strained through four layers of cheesecloth, mixed in equal proportions, and kept in a water bath at 39°C (Theodorou et al., 1994; Mauricio et al., 1999). The pH of rumen liquid was  $6.7 \pm 0.2$ , which was measured with a potentiometer (HI 991002, Hanna Instruments, Woonsocket, RI, USA).

#### *2.2.1 Buffer medium*

The buffer medium was prepared in proportions according to Menke and Steingass (1988), using deionized water instead of distilled water. The buffer medium was a mixture of macro and microminerals solutions, artificial saliva, reducing solution, and resazurin. All reagents were from Sigma-Aldrich, Milwaukee, WI, USA.

### *2.2.3 Preparation of substrate for fermentation in vitro*

Substrates were prepared to determinate the kinetics of gas production according to *in vitro* methodology described by Theodorou et al. (1994). Four hundred milligrams of substrate were placed in a 125-ml serum bottle (Wheaton, Boston, MA, USA), with the addition of 40 ml of buffer. Serum bottles were flushed with CO<sub>2</sub> and sealed with 20 mm butyl rubber stoppers and an aluminum crimp cape (Wheaton, Boston, USA). Serum bottles with substrate and buffer medium were stored overnight at 4°C. During the next morning, the serum bottles were warmed in an incubator at 39°C and then injected with 20 ml of rumen liquid using a 18 gauge needle. The displaced gas was allowed to escape prior to removing the needle. The serum bottles were shaken and then placed in the incubator (Mauricio et al., 1999). Blanks were used to correct for gas release and residual fermentation resulting directly from the inoculum and buffer medium. Substrates and blanks were run twice per treatment (experimental units), each run was conducted on a different day with three serum bottles (measurement units) per treatment.

### *2.2.4 Readings of gas production*

Gas pressure measurements (psi unit) were made with a USB output pressure transducer (PX409-015GUSBH, Omega Engineering Inc., Stamford, CT, USA) at 2, 4, 6, 8, 12, 18, 24, 36, 48, and 72 h of incubation (Experiment 1), or at 2, 4, 6, 8, 12, 18, 24, 36, 48 h (Experiments 2 to 4). The transducer was connected to a 23-gauge needle and the needle was inserted through the butyl rubber stopper to read the gas pressure (Theodorou et al., 1994). After taking the reading, the transducer was unplugged from the needle, which continued to be inserted in the rubber stopper until the headspace gas

pressure returned to ambient pressure (Mauricio et al., 1999). Serum bottles were shaken and returned to their position in the incubator (Theodorou et al., 1994). Pressure readings were taken in the same order that bottles were injected with rumen fluid. Fermentation was stopped after the last reading by placing the serum bottles into a fridge at 4°C.

The digestion residues were collected by vacuum filtration through pre-weighted filter paper of 11-µm of pore size (Whatman, Kent, England). Residues were dried at 60°C for 48 h to obtain values of DM.

### 2.3 Fermentation kinetic curve

Gas pressure values were transformed to gas volume using the equation reported by Frutos et al. (2002; Equation 1). Gas production kinetics was parameterized using the Groot et al. (1996)'s single phasic model (Equation 2) and fitted by minimum sums of squares.

$$\text{Gas volume (ml)} = 5.3407 * \text{gas pressure (psi)}. \quad (1)$$

$$G = \sum_{i=1}^n \frac{A_i}{1 + \frac{B_i}{t^{C_i}}} \quad (2)$$

where  $G$  (ml/g DOM) denotes the amount of gas produced per gram of DOM incubated, at time  $t$  after the beginning of the incubation;  $A_i$  (ml g DOM) represents the asymptotic gas production;  $B_i$  (h) is the time after starting incubation at which half of the asymptotic amount of gas has been formed;  $C_i$  is a constant determining the sharpness of the switching characteristics of the curve, as the value of  $C$  increases, the curve becomes sigmoidal with increasing slope, and  $i$  indicates the number of phases in the profile ( $i = 1$ )

(Groot et al., 1996). Both parameter *B* and *C* indicate the fermentation rate of the substrates.

#### *2.4 Chemical analyses*

Feedstuffs were analyzed for crude protein (CP), neutral (NDF) and acid (ADF) detergent fiber, ash, acid-insoluble ash (AIA), and silica content. Ash content was measured in the fermentation residues.

Crude protein was calculated by measuring the N content of the samples (Wiles et al., 1998) and then by multiplying this concentration by 6.25. Neutral detergent fiber and ADF were measured according to Van Soest et al. (1991). Ash content was obtained by burning samples at 550°C for 6 h (Allen, 1989) and the percentage of OM was calculated (OM= DM – ash). Acid-insoluble ash is an approximation of silica content (i.e., > 90% of AIA is silica [Arcos et al., 2007; Charca et al., 2007]) and it was determined by the method of 2*N* HCl (Van Keulen and Young, 1977). Silica is quantitatively recovered in the ADF residues (Van Soest, 1994). Thus, ADF residues were incinerated to measure silica content in the samples (550°C for 6 h).

#### *2.5 Statistical analyses*

Digestible DM, DOM, and fermentation kinetics parameters (*A*, *B*, and *C*; Equation 2) for medusahead harvested at different maturity stages, anatomical parts of M3, AH, FH, CaP, and YC (Experiment 1) were computed using a one-way analysis of variance (ANOVA) with type of substrate as the main factor. Digestible DM, DOM, and fermentation kinetics parameters of M2, AH, and FH at four different particle sizes (Experiment 2) were computed using a two-way ANOVA with forage species, particle

size, and their interaction as the main factors. Linear regressions were performed to test the relationship between particle size and digestibility of each forage species. Digestible DM, DOM, and fermentation kinetics parameters of a) mixtures of M2:CaP, b) mixtures of M2:AH (Experiment 3), and c) mixtures of SiO<sub>2</sub> and AH (Experiment 4) were computed using a one-way ANOVA with mixture rate as the main factor. The DDM, DOM, and gas production parameters in the mixtures (M2:CaP, M2:AH, and AH:SiO<sub>2</sub>) were estimated from values obtained from pure substrates and then compared to the observed values using one-way ANOVAs. The model diagnostics included testing for a normal distribution and homoscedasticity, and transformed when needed according to the Box-Cox method; non-transformed means  $\pm$  standard error of the mean (SEM) are reported. Means were analyzed using Tukey's multiple comparison test when *F*-ratios were significant ( $P < 0.05$ ). A tendency was considered when  $0.05 < P < 0.15$ . Analyses were computed using SAS (SAS Inst., Inc. Cary, NC; Version 9.4 for Windows).

### 3. Results

#### 3.1 Chemical composition of substrates

Chemical composition of the substrates assayed is shown in Table 4-1. Both concentrates and TF had a moderate content of silica. AH and FH were the substrates with the greatest content of CP. Medusahead declined in nutritional quality with plant maturity, i.e., M1 showed greater content of CP and lower content of fiber and silica than M3. Medusahead thatch had the greatest content of ADF and silica. Inflorescences in medusahead (M3I) showed better nutritional quality than the whole plant (M3) or leaves + stems (M3L).

### *3.2 Experiment 1: Digestibility of medusahead at different phenological stages relative to that of other forages and concentrates*

#### *3.2.1 Digestibility of dry and organic matter*

Substrates had different DDM after 72-h of incubation ( $P < 0.0001$ ). Both feed concentrates (CaP and YC) were more digestible than M3L, M4, T, and AH ( $P < 0.05$ ; Table 4-2). Feed concentrates, M1-M3, M3I, and FH shared similar DDM values ( $P > 0.05$ ; Table 4-2); with feed concentrates tending to display greater digestibility values than M3 ( $P = 0.0614$  and  $P = 0.0513$ , respectively). AH and T showed the lowest values of DDM ( $P < 0.01$ ), with value for T being lower than that recorded for AH ( $P = 0.0001$ ). Digestibility estimates did not differ among any of the different medusahead maturity stages assayed from the same growing season ( $P > 0.05$ ), despite a numerical decrease in digestibility from the vegetative (M1) to the senescent stage (M4; Table 4-2). Leaves + stems in M3 (M3L) were less digestible than inflorescences (M3I;  $P = 0.0284$ ) (Table 4-2).

Values for DOM were different between substrates ( $P < 0.0001$ ) and showed similar patterns to those described for DDM (Table 4-2). Medusahead substrates, except thatch, had greater ( $P < 0.05$ ) DOM than alfalfa hay and similar DOM to tall fescue hay ( $P > 0.05$ ). The youngest stage of medusahead assayed (M1) had similar DOM than feed concentrates ( $P > 0.05$ ); however, M1 tended to show lower DOM values than the CaP supplement ( $P = 0.0817$ ).

#### *3.2.2 Fermentation Kinetics*

The asymptotic gas production (A parameter) was different between substrates ( $P = 0.0388$ ). The AH substrate produced less gas than the two most mature medusahead

samples (M4 and T;  $P < 0.05$ ; Table 4-2), and tended to produce less gas than M3 and M3L ( $P = 0.1434$  and  $P = 0.1236$ , respectively).

Differences were also detected in the time it took the incubation of different substrates to reach half the amount of gas produced ( $B$  parameter) during the 72-h fermentation period ( $P < 0.0001$ ). It took a shorter time for the AH inoculum to reach the  $B$  value than for the rest of the assayed substrates (AH vs. M1;  $P = 0.0581$ ; AH vs. rest of the substrates;  $P < 0.05$ ; Table 4-2). In contrast, thatch was the substrate with the greatest  $B$  value ( $P < 0.05$ ; Table 4-2). Samples from both concentrates (CaP and YC) and medusahead in the late vegetative (M1) and mid reproductive (M2) stages showed similar  $B$  values ( $P > 0.05$ ; Table 4-2), whereas M3 tended to show lower  $B$  values than M4 ( $P = 0.0915$ ).

Curves of gas production presented different shape ( $C$  values;  $P < 0.0001$ ). Both concentrates (CaP and YC), and AH showed the greatest  $C$  values, suggesting high rates of fermentation ( $P < 0.05$ ; Table 4-2) and YC tended to have greater slope than AH ( $P = 0.0797$ ). The lowest values for  $C$  were observed in the most mature stages of medusahead from the same growing season (M3 and M4;  $P < 0.05$ ; Table 4-2). M2 tended to have a greater  $C$  value than FH ( $P = 0.0888$ ) and FH tended to have a greater  $C$  value than M4 ( $P = 0.1349$ ).

### *3.3 Experiment 2: Digestibility of substrates at different particle sizes*

#### *3.3.1 Digestibility of dry and organic matter*

Values of DDM and DOM for each plant species at different particle sizes after 48-h of incubation are shown in Table 4-2. Plant species ( $P = 0.0327$  and  $P = 0.0046$ ) and



particle size ( $P = 0.0017$  and  $P = 0.0147$ ) had an effect on DDM and DOM, respectively, with no interaction between plant species and particle size ( $P > 0.05$ ). Comparing the three plants species, medusahead had greater DDM ( $P = 0.0371$ ) and DOM ( $P = 0.0035$ ) than AH and FH tended to show greater DDM than AH ( $P = 0.0835$ ).

Regarding particle size of the three plant species, particles of 1 mm showed greater DDM and DOM than particles of 20 mm ( $P = 0.0012$  and  $P = 0.0102$ , respectively). Particles of 1 mm also showed greater DDM than particles of 10 mm ( $P = 0.0139$ ) and tended to be greater on an OM basis ( $P = 0.0732$ ).

When looking at the linear relationship between digestibility and particle size, greater particle sizes led to lower DDM for AH and medusahead (Fig. 4-1A). An increment in particle size did not have effect on OM digestibility of AH and FH, but it reduced DOM of medusahead ( $P < 0.05$ ; Fig. 4-1B).

### 3.3.2 Fermentation kinetics

The parameters  $A$ ,  $B$ , and  $C$  of fermentation kinetics for the forage species of different particle sizes are depicted in Table 4-2. Significant effects were detected for forage species ( $P < 0.0001$ ), particle size ( $P < 0.0001$ ), and their interaction ( $P < 0.03$ ). Medusahead and FH had greater asymptotic ( $A$  parameter) values than AH ( $P < 0.0001$ ). Comparing particle sizes, 1-mm particle size had the lowest asymptotic value than any other particle lengths ( $P < 0.0001$ ).

AH showed the shortest time to reach half the amount of total gas production ( $B$  parameter) ( $P < 0.0001$ ) and the smallest particles (1 mm) also led to the smallest values of  $B$  ( $P < 0.0001$ ). All forage species had different values of  $C$  ( $P < 0.0001$ ); with the greatest and lowest values being recorded for AH ( $P < 0.0001$ ) and FH ( $P < 0.0001$ ),

respectively. Particle size also had effect on the  $C$  parameter ( $P < 0.0001$ ); with particles of 1 mm showing the greatest  $C$  values ( $P < 0.0001$ ).

Alfalfa hay (AH) of 1, 5, and 10 mm particle size shared similar values of gas production ( $P > 0.05$ ; Table 4-2), but AH of 1 mm tended to produce less gas than AH of 5 mm ( $P = 0.0886$ ). Medusahead and FH ground to 1 mm, and AH cut to particles  $\geq 5$  mm showed no difference in gas production ( $P > 0.05$ ), but FH ground to 1-mm tended to produce more gas than AH particles cut to 10-mm ( $P = 0.0720$ ).

The time to reach half the amount of total gas production ( $B$ ) was the shortest for 1-mm AH particles ( $P < 0.05$ ; Table 4-2). Medusahead and FH ground to 1 mm, and AH cut to 20 mm had similar  $B$  values ( $P > 0.05$ ; Table 4-2).

Plant species at different particle sizes could be clustered according to the  $C$  parameter. All particle sizes of AH and medusahead ground to 1 mm had greater slopes than any other substrate ( $P < 0.05$ ; Table 4-2). FH of 1 mm and medusahead  $\geq 5$  mm had similar slopes ( $P > 0.05$ ; Table 4-2). FH particles  $\geq 5$  mm did not show differences between their curves'  $C$  values ( $P > 0.05$ ; Table 4-2).

### *3.4 Experiment 3: Digestibility of medusahead mixtures – Associative effects*

#### *3.4.1 Digestibility of dry and organic matter*

Both digestibility of DM and OM of the assayed mixtures are depicted in Fig. 4-2. Mixing medusahead with the concentrate (CaP) did not have any influence on DDM ( $P = 0.7967$ ) or DOM ( $P = 0.1552$ ) (Fig. 4-2A).

Mixtures of medusahead with AH had different DOM ( $P < 0.0211$ ); pure medusahead had greater DOM than pure AH ( $P = 0.0291$ ) (Fig. 4-2B). Mixtures with

90% of medusahead tended to have greater DOM than pure AH ( $P = 0.0524$ ), and pure medusahead tended to have greater DOM than the 50:50 medusahead:AH mixture ( $P = 0.0553$ ). Mixtures of medusahead with AH also had different DDM ( $P = 0.0368$ ), however the *post hoc* test did not show significant differences between mixtures (Fig. 4-2B), showing just tendencies. Pure medusahead tended to have greater DDM than pure AH ( $P = 0.0536$ ) and the 50:50 mixture ( $P = 0.0813$ ), whereas the 90:10 mixture tended to show greater digestibility than pure AH ( $P = 0.0943$ ).

No significant differences in digestibility were detected when comparing observed values for the mixtures vs. estimated values from single substrates. Only the DOM ( $P = 0.1083$ ) and DDM ( $P = 0.1165$ ) of the observed 50:50 mix medusahead:AH (Fig. 4-2) tended to be lower than the estimated values from single substrates (DOM=  $586 \pm 16$  g/kg OM; DDM=  $562 \pm 15$  g/kg DM).

### 3.4.2 Fermentation kinetics

Gas production curves and parameters of medusahead:CaP mixtures are shown in Fig. 4-3A. Medusahead:CaP mixtures had an influence on parameter *A* ( $P = 0.0379$ ). Pure CaP produced less gas than pure medusahead and the 90:10 mixture ( $P < 0.05$ ), and tended to produce less gas than the 50:50 mixture ( $P = 0.1295$ ).

The parameter *B* was not affected for different mixtures ( $P = 0.2617$ ). However, different mixtures had an effect on the shape of the curves (*C* parameter;  $P = 0.0011$ ). Pure CaP had the greatest *C* value ( $P < 0.05$ ), whereas the 50:50 mixture had a greater *C* value than pure medusahead ( $P = 0.0297$ ) and tended to be greater than the 90:10 mixture ( $P = 0.0648$ ).

Fermentation curves and parameters of medusahead:AH mixtures are shown in Fig. 4-3B. Mixtures influenced the parameter  $A$  ( $P = 0.0506$ ) and  $B$  ( $P = 0.0444$ ), and tended to have effect on parameter  $C$  ( $P = 0.0797$ ). Pure alfalfa hay produced a smaller amount of gas ( $A$ ) than pure medusahead ( $P = 0.0377$ ), and tended to produce a smaller amount of gas than the 90:10 and 50:50 mixtures ( $P = 0.1114$  and  $P = 0.1209$ , respectively).

Pure AH had a lower  $B$  value than pure medusahead ( $P = 0.0378$ ) and tended to show a lower value than the 70:30 mix ( $P = 0.0806$ ). Parameter  $C$  tended to be greater in pure AH than in the 50:50 and 70:30 mixtures ( $P = 0.0679$  and  $P = 0.1224$ , respectively).

Comparing observed values with calculated values, the observed asymptotic parameter ( $A$ ; Figs. 4-3A, B) tended to be greater than the calculated  $A$  parameter in both medusahead:CaP ( $P = 0.0861$ ) and medusahead:AH ( $P = 0.0598$ ) 50:50 mixtures. The calculated value for medusahead:CaP was  $386.6 \pm 3.4$  ml of gas/g DOM and for medusahead:AH was  $375.5 \pm 10.2$  ml of gas /g DOM.

### *3.5 Experiment 4: Effect of soluble silica on alfalfa hay digestibility*

Values of digestibility and curves of fermentation kinetics are shown in Figs. 4-2C and 4-3C, respectively. Silica tended to have an effect in DM digestibility ( $P = 0.0701$ ): AH with 5.0%  $\text{SiO}_2$  tended to have lower digestibility than AH with 2.5%  $\text{SiO}_2$  ( $P = 0.0842$ ) and pure AH ( $P = 0.0952$ ) (Fig. 4-2C). Different amounts of soluble silica did not have effect on DOM ( $P = 0.6813$ , Fig. 4-2C).

Parameters  $A$  ( $P = 0.3848$ ) and  $B$  ( $P = 0.2651$ ) of the gas production curve were not affected by the addition of  $\text{SiO}_2$  (Fig. 4-3C). Addition of soluble silica tended to have

an effect on parameter  $C$  ( $P = 0.1238$ ): AH with 2.5% of  $\text{SiO}_2$  tended to have a more sigmoid curve with a greater slope than pure AH ( $P = 0.1120$ ).

The observed value of  $B$  in AH with 5.0%  $\text{SiO}_2$  (Fig. 4-3C) was greater (more time to reach the half of the total gas production) than the estimated value ( $9.0 \pm 0.2$  h;  $P = 0.0465$ ), and the  $C$  parameter of AH with 2.5%  $\text{SiO}_2$  tended to have a greater observed value (a more sigmoid curve and a greater slope; Fig. 4-3C) than the calculated value ( $1.579 \pm 0.008$ ;  $P = 0.1061$ ).

## 4. Discussion

### 4.1 Medusahead digestibility and nutritional value relative to other feeds

Consistent with prior research (e.g., Van Soest and Jones, 1968; Kenney and Black, 1984), our results show that the apparent digestibility of alfalfa was lower than that of grasses, including medusahead. Digestibility values of medusahead found in this study were consistent with values ( $> 65\%$ ) reported for *T. caput-medusae* (unknown ssp.) by Shawrang and Nikkhah (2005). These values of digestibility are relatively high when considering that medusahead contains high content of silica, an antinutritional factor in forages (Lusk et al., 1961; Young, 1992).

Van Soest and Jones (1968) and Smith et al. (1971) estimated for grasses that there is a decrease in one to three units of DM digestibility per unit of increase in silica content. We did not find a significantly reduction in DM and OM digestibility for medusahead (ground at 1 mm of particle size) with increments in silica content, which occurred as medusahead matured from the late vegetative stage to the late reproductive stage. Additionally, tall fescue hay and medusahead at the mid reproductive stage had

similar digestibilities of DM and OM, but medusahead had 59% more silica content than tall fescue hay. This suggests that the decline in digestibility due to silica content may not always be proportional to a 3:1 ratio as suggested by Van Soest and Jones (1968). More recent studies also support this notion as Van Soest (1993) reports additional studies for grasses where silica did not affect digestibility.

The relatively high values of apparent digestibility found in medusahead are in stark contrast with intake values reported for this weed (e.g., Hamilton et al., 2015; Villalba and Burritt, 2015). In fact, the ground substrate collected at different phenological stages of medusahead, except thatch, led to values of DM and OM digestibility similar to those found in concentrates. Medusahead intake by livestock is typically low at all stages of maturity during the growing season (Lusk et al., 1961; Hamilton et al., 2015; Villalba and Burritt, 2015), even when consumption of the weed by sheep is greater in younger than in older plants (Hamilton et al., 2015). In general, as reported in this study, fiber content is lower and protein content greater in younger medusahead plants. In addition, the inflorescence in medusahead showed greater CP content and digestibility values than those found in the whole plant. Consistent with these findings, lambs display greater preference for inflorescences than for leaves and stems when offered in a choice test (Villalba and Burritt, 2015).

In contrast to plants from the current growing season, the low digestibility of thatch matches its low intake by lambs (Hamilton et al., 2015); although, lambs in a previous study ate more thatch than medusahead in the reproductive phenological stage (Hamilton et al., 2015). This pattern may be explained by the fact that thatch in the present study had greater AIA and lower NDF and ADF contents than those reported by

Hamilton et al. (2015). The silica content in plant tissues varies according to availability of soluble silica in the seedbed (Massey and Hartley, 2006) and to the evaporation rate of different plant tissues (Raven, 2003).

#### *4.2 Fermentation kinetics and intake*

The voluntary DM intake of roughage by ruminants has a direct correlation with gas production between 2 and 8 h of *in vitro* incubation (e.g., related to the fermentation of feed solubles), and with low amounts of gas production at later times of incubation (e.g., related to fermentation of structural carbohydrates) (Blümmel et al., 2005). Therefore, the more gas produced at the beginning of fermentation, the greater the value of voluntary intake, which agrees with the relatively small values for the parameter *B* found in alfalfa (the time it took the incubation of alfalfa to reach half the total amount of gas produced during fermentation). In contrast, medusahead had a slower rate of fermentation (longer time to reach half the amount of gas production), which became much slower as medusahead matured. For example, substrate from medusahead collected at mid reproductive stage required 72% more time to reach half the amount of gas produced than medusahead harvested at the vegetative stage with the same total amount of gas produced or asymptotic value (parameter *A*) for both substrates. Furthermore, the fermentation of medusahead was extended over time relative to more palatable forages and concentrates. The greater gas production observed for medusahead after 24 hours of incubation was due to fermentation of structural carbohydrates, which can be seen at different rates in different forages with different types and amounts of cell wall (Blümmel et al., 2005). In addition, the structure of plant cell walls can change with maturity and as a consequence impact forage digestibility. For instance, Van Dyne (1962) found that

isolated cellulose from immature medusahead plants had greater *in vitro* digestibility (79-82%) than cellulose from mature medusahead (70-72%), mature mixed annual rangeland (67%), and alfalfa (51%).

Digestibility of rice straw (rich in silica) is limited to damaged regions of the cuticle and edges of feed particles (Bae et al., 1997). This limitation likely occurs in medusahead, suggesting that the slow fermentation rate observed was caused by the slow exposure and release of structural and soluble fermentable compounds induced by medusahead's physical characteristics. Medusahead has smaller stems and leaves than tall fescue and presents long silicified awns (Swenson et al., 1964; Stubbendreck et al., 2003). Thus, medusahead should have more silicified epidermis than the same amount (on a weight or volume basis) of tall fescue. Furthermore, larger edges in chewed leaves and empty stems in tall fescue should give more unprotected area for bacterial colonization and degradation. This may explain why lambs eat more and show greater preference for tall fescue than for medusahead (Villalba and Burritt, 2015), even when both grasses have similar digestibilities. This is why we hypothesized that particle size reduction may play a significant role at explaining medusahead intake and palatability.

#### *4.3 Fermentation kinetics and particle size*

Particle size may have a significant impact on medusahead digestion given that silica is present on the epidermis (Swenson et al., 1964), acting as a physical barrier (Van Soest and Jones, 1968; Mayland and Shewmaker, 2001). This prevents microbial attachment and/or colonization of plant cell walls and thus enzymatic degradation of structural carbohydrates, an effect less evident in tall fescue with moderate content of silica.



The rate of digestion depends on reduction of particle size and the increment of “unprotected” areas by silica that allows for greater surface area and thus more access to colonizing rumen bacteria. In turn, particle size reduction depends on rumination rate and resistance to fracture of the substrate (Van Soest, 1994). Considering that chewing is probably the major force in reducing particle size (Van Soest, 1994; Allen, 1996), and the effect of cell wall composition (i.e., cellulose and lignin) on forage breakup, alfalfa may shatter into shorter particles, while grasses might break into more needle-like particles (Van Soest, 1994). Furthermore, silica can decrease the mechanical disruption of the chlorenchyma cell walls in grasses, which contain high levels of soluble carbohydrates and protein (Hunt et al., 2008).

*In vitro* and *in sacco* digestibility trials have the limitation of not being able to evaluate the rate of mechanical reduction of particle size since substrates are typically ground to 1mm (Menke and Steingass, 1989; Theodorou et al., 1994). This limitation is key as particle size reduction is of major importance in the alleviation of rumen fill and, consequently, has a significant impact on feed intake (Allen, 1996). Welch (1967) found that wethers showed a 30% reduction in the voluntary DM intake of chopped alfalfa hay when 150 g of 7-cm long polypropylene fibers were inserted into the reticulorumen. Intake decreased immediately after insertion of the fibers and gradually increased as the fibers were reduced in size by rumination and passed through the digestive tract. Therefore, medusahead with its inherent chemical (i.e., silica and lignin) and anatomical characteristics might have a similar fill effect and reduction of passage rate, requiring more time and energy for chewing and reducing the medusahead particles to increase the area exposed to microbial activity. Consistent with these ideas, we found a stronger

impact of particle size on digestibility of medusahead than on the digestibility of forages (i.e., alfalfa and tall fescue hay) that typically lead to greater intake values by livestock. For instance, particle size did not have an influence on DOM in alfalfa, but greater particles inhibited DOM and DDM in medusahead. When looking at the linear relationship between DOM and particle size, only significant effects were detected for medusahead.

Palatability is a complex process that integrates odor, taste, and texture with the post-ingestive effects of nutrients and toxins (Provenza, 1995). Gustatory, olfactory, and tactile sensory receptors interact with the visceral receptors that respond to nutrients and toxins (chemo-receptors), osmolality (osmo-receptors), and distension (mechano-receptors). Collectively, these neurally mediated sensorial–digestive process interactions enable animals to discriminate among foods, each of which possesses a distinct utility, and they encourage animals to eat a variety of foods and to forage in a variety of locations (Provenza et al., 2003). Aversions become pronounced when foods contain high levels of toxins, low level of nutrients or nutrient imbalances (Provenza et al., 2003). Medusahead does not cause aversion in lambs (Hamilton et al., 2015). However, the slow particle size reduction in medusahead may result in a slow release of nutrients and energy from this weed, which will negatively influence preferences and intake.

#### *4.4 Fermentation of medusahead mixtures*

The addition of alfalfa hay or the energy concentrate to medusahead did not lead to positive associative effects as expected. Digestibility values of the mixes were in general a linear combination of the digestibilities found in the pure substrates. This result is consistent with the fact that supplementation programs have weak or non-significant

effects on medusahead intake and preference (Hamilton et al., 2015; Villalba and Burritt, 2015).

On the other hand, medusahead mixed with alfalfa or CaP did not cause significant negative associative effects on digestibility. It is expected that the silicified epidermis of medusahead just affects the digestibility of the plant's own constituents. However, soluble silica also reduces digestibility (Smith and Nelson, 1975; Shimojo and Goto, 1989), and the presence of this compound could be greater in young plants and leaves with more photosynthetic activity and evapotranspiration, physiological processes associated with a greater absorption of orthosilicic acid (soluble silica) by the plant's roots. Thus, it is likely that young medusahead plants have a greater negative impact on associative effects through soluble silica than mature plants with a greater content of amorphous silica in their tissues.

#### *4.5 Effect of soluble silica in alfalfa hay digestibility*

Smith and Nelson (1975), and Shimojo and Goto (1989) found that soluble silica decreases final *in vitro* digestibility in grasses, but not in alfalfa (Smith and Nelson, 1975). A similar result was found in this study with the addition of SiO<sub>2</sub> to alfalfa hay. Soluble silica has the capacity to adsorb cations (i.e., Ca and Mn) producing insoluble complexes and thus reduce their absorption by ruminants (Prabowo and Spears, 1992). It is also likely that silica reduces the availability of Ca to rumen microorganisms and thus inhibits forage digestion since microorganisms need Ca to colonize substrates and start the digestion of structural carbohydrates (Pell and Schofield, 1993). Alfalfa hay has a high Ca content (NRC, 2007) that could keep free Ca for microorganism to use, reducing the negative effects of dissolved silica in the rumen fluid. This complexation likely

happens with other cations (Ehrlich et al., 2010), which is supported by the fact that the addition of minerals (Mg, Mn, Zn, Co, and Cu) to the incubation medium suppresses the inhibitory effects of soluble silica on *in vitro* grass digestibility (Smith and Nelson, 1975).

Fermentation of alfalfa hay + SiO<sub>2</sub> in the present study tended to be more sigmoid than pure alfalfa which suggests less gas production at the initial hours of fermentation, with reductions of 20-23%, 17-20%, 15-18%, and 14-16% for 2, 4, 6, and 8 h of incubation with 2.5 and 5% SiO<sub>2</sub>, respectively. This reduction in gas production at initial hours of fermentation was confirmed by the longer time it took for the alfalfa hay + 5% SiO<sub>2</sub> substrate to reach half the amount of gas production (observed value) than the calculated value from the pure substrate. Alfalfa is well known for its high protein content (NRC, 2007) and SiO<sub>2</sub> likely reduces the rate of nitrogen use by rumen microorganism since silica binds to positively charged amino acids, peptides, and proteins (Kauss et al., 2003; Currie and Perry, 2007; Neethirajan et al., 2009), as well as digestive enzymes (Kind et al., 1954; Shimojo and Goto, 1989). Kauss et al. (2003) reported that the amount of silica precipitated was proportional to the amount of peptides present in the *in vitro* medium. In laboratory assays, hydrogen bonds and other polar interactions (ion-dipole, dipole-dipole, and van-der-Waals), occur between peptides and silanol (Si-OH)/siloxides (Si-O<sup>-</sup>) groups (Patwardhan et al., 2012). Furthermore, silica-peptide complexes are formed according to physical characteristics of amorphous silica (i.e., degree of ionization of surface and size; Patwardhan et al., 2012), to the kind and content of peptides, and to pH, with greater affinities at pH between 6-7 (Kauss et al. 2003). It is worth mentioning that cellulolytic microorganisms grow optimally at pH 6.7

$\pm 0.5$  (Van Soest, 1994). In addition, Shimojo and Goto (1989) suggested that the effect of soluble silica on digestion in different tissues of a grass could be negatively associated to energy and protein content.

In summary, addition of silica to ground alfalfa reduced gas production during the first hours of digestion. This suggests that the inhibitory effects of silica on digestibility may not only be mechanical (e.g., the creation of a varnish that constraints bacterial colonization and particle size reduction) but also chemical (e.g., inhibition of enzymes, unavailability of free positively charged molecules and ions).

## 5. Conclusions

Despite the constraint of containing high contents of silica, an antinutritional factor, the apparent digestibility of medusahead was comparable to values observed in more palatable substrates (tall fescue hay, energy concentrates) and even greater than values observed in a palatable legume (alfalfa). This pattern is not in line with the typical low intake and palatability of medusahead. This discrepancy may be explained by the fact that *in vitro* assays use finely ground plant material which prevent estimating the influence of particle size in the digestion process and the potential (mechanical) inhibitory effects of silica. The high silica content of the weed forms an epidermal varnish, which decreases bacterial attachment and the enzymatic breakdown of plant material in the rumen, decreasing passage rate and prolonging fill effects that reduce intake and palatability. By using particles of different sizes our study revealed a much greater inhibitory effect of particle size on digestibility for medusahead than for the rest of the forages assayed. In addition, fermentation kinetics of medusahead showed a delayed gas production relative to more palatable forages like alfalfa, another factor

explaining the low palatability of the weed. No associative effects were found by mixing medusahead with alfalfa or with an energy concentrate, a finding which is in line with the marginal or nil effects of supplements on medusahead intake reported in *in vivo* studies. Finally, addition of soluble silica to ground alfalfa revealed a reduced speed of gas production, suggesting that the effects of silica at reducing forage digestibility may not only be mechanical but also chemical (e.g., inhibition of enzymatic activity in the rumen fluid).

### **Conflict of interest**

The Authors have no conflict of interest with this manuscript.

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**Table 4-1**Chemical composition (mean  $\pm$  SD) of the feeds and forages assayed (g/kg dry matter).

Feedstuff	CP	NDF	ADF	Ash	AIA	Silica
Medusahead at different phenological stages						
Late vegetative, M1	124 $\pm$ 0	562 $\pm$ 4	315 $\pm$ 6	124 $\pm$ 0	70 $\pm$ 0	64 $\pm$ 0
Mid reproductive, M2	102 $\pm$ 1	645 $\pm$ 11	362 $\pm$ 3	112 $\pm$ 1	66 $\pm$ 1	69 $\pm$ 2
Late reproductive						
Whole plant, M3	73 $\pm$ 0	651 $\pm$ 3	402 $\pm$ 5	115 $\pm$ 0	86 $\pm$ 0	83 $\pm$ 1
Inflorescence, M3I	95 $\pm$ 2	638 $\pm$ 1	369 $\pm$ 3	106 $\pm$ 0	77 $\pm$ 0	74 $\pm$ 2
Leaves + stems, M3L	53 $\pm$ 1	674 $\pm$ 8	429 $\pm$ 2	128 $\pm$ 1	93 $\pm$ 1	87 $\pm$ 1
Senescence, M4	76 $\pm$ 0	610 $\pm$ 3	365 $\pm$ 4	94 $\pm$ 1	69 $\pm$ 1	65 $\pm$ 1
Thatch, T	82 $\pm$ 1	612 $\pm$ 16	467 $\pm$ 11	164 $\pm$ 1	132 $\pm$ 0	105 $\pm$ 8
Convectional feedstuffs						
Alfalfa hay, AH	185 $\pm$ 3	465 $\pm$ 10	384 $\pm$ 8	82 $\pm$ 0	2 $\pm$ 1	9 $\pm$ 1
Tall fescue hay, FH	142 $\pm$ 0	607 $\pm$ 8	327 $\pm$ 0	142 $\pm$ 0	32 $\pm$ 0	41 $\pm$ 2
Ca-propionate concentrate, CaP	100 $\pm$ 3	320 $\pm$ 3	178 $\pm$ 3	93 $\pm$ 1	23 $\pm$ 0	28 $\pm$ 3
Yeast culture concentrate, YC	109 $\pm$ 3	353 $\pm$ 3	191 $\pm$ 7	81 $\pm$ 0	22 $\pm$ 1	30 $\pm$ 0

CP= crude protein

NDF= neutral-detergent fiber

ADF= acid-detergent fiber

AIA= acid-insoluble ash

**Table 4-2**

Apparent digestibility and fermentation kinetic curve parameters (mean  $\pm$  SEM) of medusahead at different phenological stages and conventional feedstuffs (Experiment 1), and the effect of particle size on plant digestibility (Experiment 2).

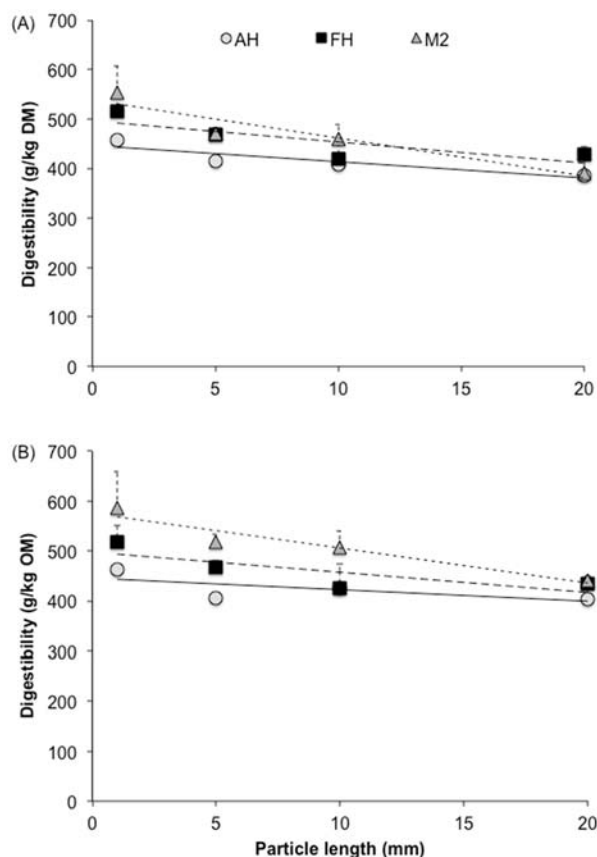
Substrate	Digestibility (g/kg)		Kinetic curve parameters (g organic matter)		
	Dry matter	Organic matter	A (ml)	B (hours)	C
Experiment 1: Digestibility of medusahead at different phenological stages, forages and concentrates					
Medusahead					
Late vegetative, M1	672 $\pm$ 17 <sup>ab</sup>	705 $\pm$ 3 <sup>abc</sup>	392.0 $\pm$ 30.1 <sup>ab</sup>	14.7 $\pm$ 0.8 <sup>e</sup>	1.33 $\pm$ 0.05 <sup>ab</sup>
Mid reproductive, M2	662 $\pm$ 22 <sup>abc</sup>	697 $\pm$ 15 <sup>bc</sup>	408.9 $\pm$ 36.9 <sup>ab</sup>	20.3 $\pm$ 1.7 <sup>de</sup>	1.46 $\pm$ 0.06 <sup>dc</sup>
Late reproductive					
Whole plant, M3	629 $\pm$ 30 <sup>abc</sup>	668 $\pm$ 20 <sup>c</sup>	443.9 $\pm$ 45.1 <sup>ab</sup>	25.4 $\pm$ 1.5 <sup>bcd</sup>	1.19 $\pm$ 0.00 <sup>bd</sup>
Inflorescence, M3I	680 $\pm$ 20 <sup>ab</sup>	719 $\pm$ 1 <sup>abc</sup>	432.6 $\pm$ 23.8 <sup>ab</sup>	26.4 $\pm$ 0.1 <sup>bcd</sup>	1.23 $\pm$ 0.01 <sup>abd</sup>
Leaves + stems, M3L	578 $\pm$ 29 <sup>cd</sup>	610 $\pm$ 25 <sup>c</sup>	451.2 $\pm$ 51.2 <sup>ab</sup>	31.9 $\pm$ 2.5 <sup>bc</sup>	1.15 $\pm$ 0.03 <sup>bd</sup>
Senescence, M4	622 $\pm$ 1 <sup>bc</sup>	648 $\pm$ 0 <sup>c</sup>	498.2 $\pm$ 29.9 <sup>a</sup>	37.0 $\pm$ 0.1 <sup>b</sup>	1.08 $\pm$ 0.00 <sup>d</sup>
Thatch, T	400 $\pm$ 3 <sup>e</sup>	459 $\pm$ 8 <sup>e</sup>	507.7 $\pm$ 130.8 <sup>a</sup>	60.4 $\pm$ 11.9 <sup>a</sup>	1.30 $\pm$ 0.10 <sup>ab</sup>
Convectional feedstuffs					
Alfalfa hay, AH	511 $\pm$ 1 <sup>d</sup>	533 $\pm$ 8 <sup>d</sup>	274.4 $\pm$ 0.0 <sup>b</sup>	9.7 $\pm$ 0.0 <sup>f</sup>	1.65 $\pm$ 0.00 <sup>ce</sup>
Tall fescue hay, FH	661 $\pm$ 3 <sup>ab</sup>	670 $\pm$ 5 <sup>c</sup>	387.3 $\pm$ 18.0 <sup>ab</sup>	21.3 $\pm$ 0.3 <sup>cde</sup>	1.24 $\pm$ 0.00 <sup>abd</sup>
Ca-propionate concentrate, CaP	735 $\pm$ 3 <sup>a</sup>	786 $\pm$ 6 <sup>a</sup>	329.6 $\pm$ 7.8 <sup>ab</sup>	15.1 $\pm$ 0.1 <sup>e</sup>	2.21 $\pm$ 0.05 <sup>f</sup>
Yeast culture concentrate, YC	739 $\pm$ 12 <sup>a</sup>	774 $\pm$ 13 <sup>ab</sup>	335.1 $\pm$ 11.7 <sup>ab</sup>	14.9 $\pm$ 0.1 <sup>e</sup>	2.10 $\pm$ 0.14 <sup>ef</sup>
Experiment 2: Digestibility of substrates at different particle sizes (mm)					
Medusahead at mid reproductive stage, M2					
1	554 $\pm$ 50	586 $\pm$ 73	493.3 $\pm$ 39.7 <sup>a</sup>	26.7 $\pm$ 1.3 <sup>ab</sup>	1.36 $\pm$ 0.01 <sup>a</sup>
5	470 $\pm$ 10	518 $\pm$ 15	1630.7 $\pm$ 590.1 <sup>b</sup>	191.5 $\pm$ 96.9 <sup>c</sup>	1.06 $\pm$ 0.06 <sup>b</sup>
10	460 $\pm$ 27	506 $\pm$ 34	2149.3 $\pm$ 471.0 <sup>b</sup>	287.7 $\pm$ 80.7 <sup>c</sup>	0.98 $\pm$ 0.03 <sup>bc</sup>
20	391 $\pm$ 5	440 $\pm$ 1	2323.9 $\pm$ 232.8 <sup>b</sup>	247.7 $\pm$ 32.0 <sup>c</sup>	0.97 $\pm$ 0.01 <sup>bc</sup>
Tall fescue hay, FH					
1	514 $\pm$ 31	518 $\pm$ 32	604.8 $\pm$ 23.2 <sup>a</sup>	33.7 $\pm$ 0.9 <sup>b</sup>	1.01 $\pm$ 0.01 <sup>b</sup>
5	469 $\pm$ 13	467 $\pm$ 13	1544.0 $\pm$ 132.0 <sup>b</sup>	208.4 $\pm$ 33.2 <sup>c</sup>	0.81 $\pm$ 0.01 <sup>cd</sup>
10	418 $\pm$ 43	426 $\pm$ 48	1410.9 $\pm$ 222.2 <sup>b</sup>	190.9 $\pm$ 42.6 <sup>c</sup>	0.85 $\pm$ 0.03 <sup>cd</sup>
20	428 $\pm$ 15	434 $\pm$ 15	1391.7 $\pm$ 117.4 <sup>b</sup>	177.2 $\pm$ 42.8 <sup>c</sup>	0.80 $\pm$ 0.01 <sup>d</sup>
Alfalfa hay, AH					
1	457 $\pm$ 3	464 $\pm$ 6	351.2 $\pm$ 13.1 <sup>c</sup>	11.3 $\pm$ 0.9 <sup>d</sup>	1.54 $\pm$ 0.03 <sup>a</sup>
5	416 $\pm$ 8	406 $\pm$ 5	451.6 $\pm$ 8.6 <sup>ac</sup>	17.5 $\pm$ 0.8 <sup>a</sup>	1.46 $\pm$ 0.02 <sup>a</sup>
10	407 $\pm$ 1	425 $\pm$ 6	431.8 $\pm$ 3.1 <sup>ac</sup>	17.0 $\pm$ 1.5 <sup>a</sup>	1.43 $\pm$ 0.07 <sup>a</sup>
20	386 $\pm$ 1	403 $\pm$ 35	488.3 $\pm$ 49.7 <sup>a</sup>	17.9 $\pm$ 0.5 <sup>a</sup>	1.41 $\pm$ 0.01 <sup>a</sup>

A represents the asymptote for the gas production curve; B is the time after starting

incubation at which half of the asymptotic amount of gas has been formed, and C is a constant determining the sharpness of the switching characteristics of the curve.

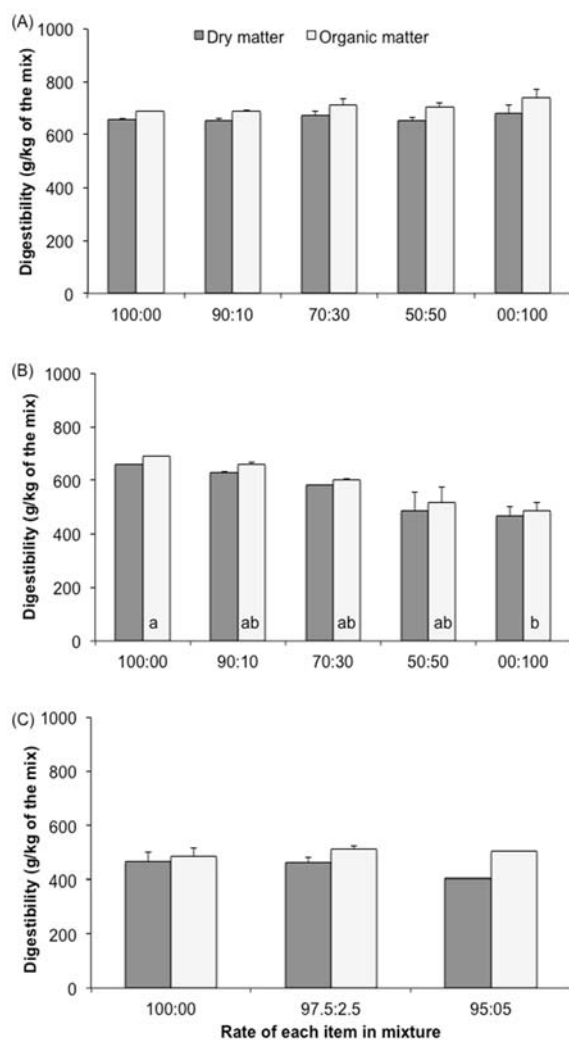
Substrates were incubated for 72 h in Experiment 1 and for 48 h in Experiment 2.

Means in a column with different characters differ ( $P < 0.05$ ).

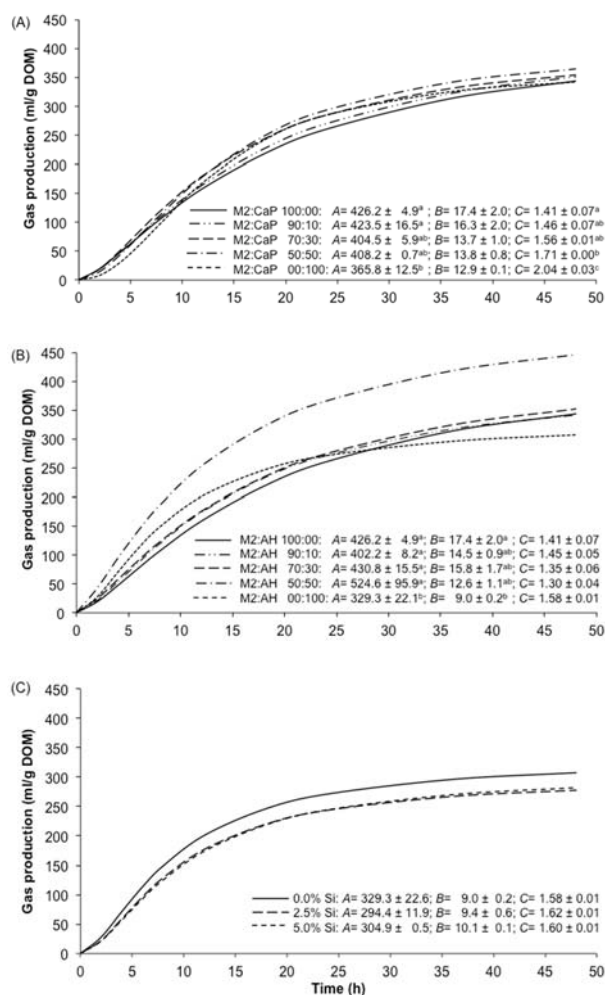


**Fig. 4-1.** Linear regressions between different particle sizes (mm) and apparent digestibility (mean  $\pm$  SEM) of alfalfa hay (AH), tall fescue hay (FH), and medusahead at the mid reproductive stage (M2). (A) Digestible dry matter (DM): AH ( $y = -0.32832x + 446.1$ ;  $R^2 = 0.59$ ;  $P = 0.025$ ); FH ( $y = -0.42557x + 495.9$ ;  $R^2 = 0.41$ ;  $P = 0.0875$ ), and M2 ( $y = -0.76871x + 538.1$ ;  $R^2 = 0.70$ ;  $P = 0.0099$ ). (B) Digestible organic matter (OM): AH ( $y = -0.76871x + 538.1$ ;  $R^2 = 0.70$ ;  $P = 0.0099$ ), FH ( $y = -0.2329x + 44.55$ ;  $R^2 = 0.31$ ;  $P = 0.1418$ ), M2 ( $y = -0.69896x + 575.6$ ;  $R^2 = 0.57$ ;  $P = 0.0306$ ). Substrate incubation was conducted for 48 h.





**Fig. 4-2.** Digestibility (mean  $\pm$  SEM) of dry matter and organic matter of different mixtures: (A) medusahead (M2):Ca-propionate concentrate (CaP); (B) medusahead (M2):alfalfa hay (AH), and (C) alfalfa hay (AH):soluble silica (SiO<sub>2</sub>). Substrate incubation was conducted for 48 h. Values with different letters are significantly different ( $P < 0.05$ ).



**Fig. 4-3.** Curves of gas production and parameters *A*, *B*, and *C* (mean ± SEM) of different mixtures with medusahead at the mid reproductive stage: (A) medusahead (M2):Ca-propionate concentrate (CaP); (B) medusahead (M2):alfalfa hay (AH), and (C) alfalfa hay (AH):soluble silica (SiO<sub>2</sub>). *A* represents the asymptotic gas production, ml/kg of digestible organic matter (DOM); *B* is the time after starting incubation at which half of the asymptotic amount of gas has been formed, and *C* is a constant determining the sharpness of the switching characteristics of the curve. Substrate incubation was conducted for 48 h. Curve parameters with different letters are significantly different ( $P < 0.05$ ).

## CHAPTER 5

## CONCLUSIONS

Medusahead is a Eurasian annual grass that due to its invasiveness and poor nutritional quality negatively impacts the structure and functioning of rangeland ecosystems (Young, 1992; Davies and Johnson, 2008). Grazing represents a sustainable and successful alternative for the control of invasive plant species (Frost and Launchbaugh, 2003; Vavra *et al.*, 2007), typically conducted with domestic sheep and goats. Nevertheless, results regarding control of medusahead through grazing have been poor and variable (James *et al.*, 2015), an outcome attributed to the low intake and palatability of this weed (Young, 1992). Despite this constraint, stakeholders in some regions of the United States list grazing as a preferred management tool to control medusahead (James *et al.*, 2015).

This study explored the influence of an energy-dense supplement on medusahead intake by ewes grazing with their lambs on medusahead-infested rangeland with a moderate level of infestation during the growing season of 2013. During the following year, the effects of early experience at grazing medusahead was tested in yearling sheep by determining the use of medusahead relative to other forages (pen trial) and to native plants on rangeland with moderate levels of medusahead infestation (grazing trial). Finally, the apparent digestibility of medusahead was assessed to understand the low intake of this weed by domestic ruminants.

The availability of an energy-dense supplement did not favor use of medusahead by ewes grazing with their lambs on medusahead-infested rangeland (Chapter 2). In general, sheep spent a low proportion of the recorded grazing events harvesting medusahead but use of the weed increased as the growing season progressed, when

almost all medusahead plants had inflorescences. A previous study shows that lambs had greater preference for medusahead inflorescences than for leaves and stems; however, the intake of inflorescences was still low in comparison to preferred high-quality feeds (Villalba and Burritt, 2015). Additionally, digestibility assays (Chapter 4) showed that medusahead inflorescences had greater digestibility than medusahead leaves and stems, thus explaining the greater preference observed for inflorescences by sheep.

A high level of similarity was found for the use of medusahead between ewes and their respective offspring; those ewes observed to graze medusahead at high frequencies had offspring that displayed a similar behavior, whereas those ewes with low to nil use of medusahead had offspring which displayed the lowest utilization of the weed (Chapter 2). This suggests that individual variation on medusahead intake is transmitted from mother to offspring, either through genetics and/or observational learning.

When lambs were weaned and released to graze medusahead-infested rangeland after a 5-day period of enclosure after weaning, they used medusahead to a similar extent to that observed before weaning (Chapter 2). Thus, experiences with mother familiarized lambs with the weed such that medusahead was no longer novel to the weaned lambs, a process that made animals more efficient at using the plant from the first day that they grazed without their mothers.

Weaned lambs matured and during the following growing season they were fed medusahead in pens with or without alfalfa hay and then they grazed medusahead-infested rangeland (Chapter 3). Early experiences with grazing medusahead and availability of alfalfa did not enhance medusahead intake relative to inexperienced and non-supplemented animals. Intake of medusahead was low and cyclic, with a more even

consumption of medusahead by experienced animals. The high contents of silica in the plant's tissues was likely responsible for the low and cyclic pattern of medusahead intake by yearlings. This may be because silica forms an epidermal varnish, which decreases bacterial attachment to plant cell walls and as a consequence reduces the enzymatic breakdown of plant material in the rumen; a process that reduces fermentation rate (Chapter 4), passage rate and that prolongs fill effects, all of which have negative impacts on feed intake by ruminants. Consistent with this view, an *in vitro* digestibility study using particles of different lengths (Chapter 4) revealed a much greater inhibitory effect of particle size on medusahead digestibility than on digestibility of other high-quality forages.

Early experience with medusahead favored weight gains and gain-to-feed ratio only when animals received medusahead in *ad libitum* amounts and then a basal diet of tall fescue hay. Experienced sheep also showed a greater intake of NaCl, which could be explained by their early experiences with grazing a plant community poor in Na. When experienced and inexperienced yearling were then transported to graze medusahead-infested rangeland, they all showed very low to nil use of medusahead, contrasting with greater values (5% of the grazing events recorded) observed the year before by the same animals. It is likely that forcing animals to ingest medusahead in pens attenuated the subsequent use of medusahead during grazing. Herbivores satiate on feeds consumed too frequently or in excess (Provenza, 1996), and the more nutritionally imbalanced the feed the greater the level of satiation (Provenza, 1996). Thus, the lack of positive post-ingestive feedback experienced by sheep consuming medusahead due to its low fermentation rates (Chapter 4) and high exposure to such low-quality feed during the pen

trial likely enhanced rejection of the weed during grazing.

When looking at the *in vitro* digestibility of medusahead - and in contrast to prior research suggesting that plants with high content of silica show low digestibility (e.g., Van Soest and Jones, 1968; Smith *et al.*, 1971) – results showed greater values than those observed for high-quality feeds such as the alfalfa hay used to feed yearlings during 2014 (Chapter 3), and similar to the digestibility of the energy-dense supplement used during the summer of 2013 (Chapter 2). This pattern is not in line with the typical low intake and palatability of medusahead. This discrepancy may be explained by the fact that *in vitro* assays use finely ground plant material which prevent estimating the influence of particle size in the digestion process and the potential (mechanical) inhibitory effects of silica. Silica forms a varnish that acts as a barrier for microbial access to the plant's cell wall. This is supported by data from the *in vitro* digestibility study using particles of different lengths (Chapter 4) which show a much greater inhibitory effect of particle size on digestibility for medusahead than for other high-quality forages. Finally, no associative effects were found by mixing medusahead with alfalfa hay or with an energy-dense supplement, a finding that is in line with the nil effects of those supplemental feeds on medusahead intake by sheep reported in Chapters 2 and 3.

In general, grazing during 2 consecutive years (experiments described in Chapters 2 and 3) reduced the abundance of medusahead, other annual grasses and bunch grasses in the plant community relative to Control plots (see Appendix). Environmental conditions, i.e., precipitation regimes above average during 2014 and 2015, played a role in the abundance of different functional groups in the community as well; an increase in annual grasses and a decline in bunch grasses and forbs was observed from 2013 to 2015

in Control ungrazed plots (Appendix). It is worth mentioning that while the abundance of medusahead in grazed plots remained below that observed in Control plots during 2015 – a year when no grazing occurred – the abundance of other annual grasses in previously grazed plots rebounded to values comparable to those observed in Control plots during 2015. This suggests that pastures need to be grazed on a yearly basis in order to keep other annual grasses (e.g., cheatgrass) at a relative lower abundance in the plant community.

Information from this Dissertation is of use for devising innovative grazing treatments aimed at combating medusahead spread in rangelands. Since sheep did not avoid medusahead when the abundance of the weed in the plant community was low (e.g., < 10% abundance), grazing treatments could be implemented to take advantage of the fact that medusahead will not increase grazing pressure on more palatable plants in the community when abundance of the weed is low. In addition, sheep displayed greater use of medusahead as the season progressed, likely due to the greater quality of the immature inflorescences (Chapter 2). This pattern may reduce the deposition of senescent medusahead matter on the soil, a process associated with the increment of fire frequency on rangelands and with the creation of an environment which favors a seedbed for medusahead seeds (Hironaka and Sindelar, 1973; Young, 1992). Grazing treatments may be implemented in a rotational system across paddocks and for consecutive years in order to reduce medusahead abundance and the accumulation of thatch on the soil surface.

Results from this Dissertation also suggest that lambs could be weaned during the growing season and that they will continue harvesting medusahead at similar rates to those observed before weaning. This process will increase efficiency as lambs will not

need to learn by trial and error (Provenza and Balph, 1987). In addition, trial and error may lengthen the process by which inexperienced sheep start consuming the weed to their capacity and in the process the quality of medusahead will decline and the plant will become stiffer, thus reducing utilization and potentially increasing the likelihood of plants dropping seeds to the ground.

For rangelands with greater levels of infestation, experienced animals may be managed to grazing cycles where they are concentrated in medusahead-infested patches for a short period of time (i.e., 1 day), moved to a diverse vegetation for 3-7 days, and then returned to medusahead patches for another short period of time. This rotation will make a more efficient use of the weed, as it will mimic the described cyclic pattern of medusahead intake by sheep exposed to *ad libitum* amounts of this weed. In addition, experienced animals in this context may use medusahead more evenly and display better gains and efficiencies than inexperienced animals. Nevertheless, it is worth mentioning that the proposed management approach should be avoided during the period of seed disarticulation, thus preventing the potential long-distance dispersal of medusahead seed via epizoochory.

Finally, the reported high correlation regarding use of medusahead between mothers and their offspring could be used to select females, either by observation and/or genetic markers, which display a high use of medusahead in order to establish flocks with the ability to harvest greater amounts of this weed than the average flock. This selection will increase the effectiveness and efficiency of targeted grazing treatments, allowing for the control of areas with greater levels of medusahead infestation.



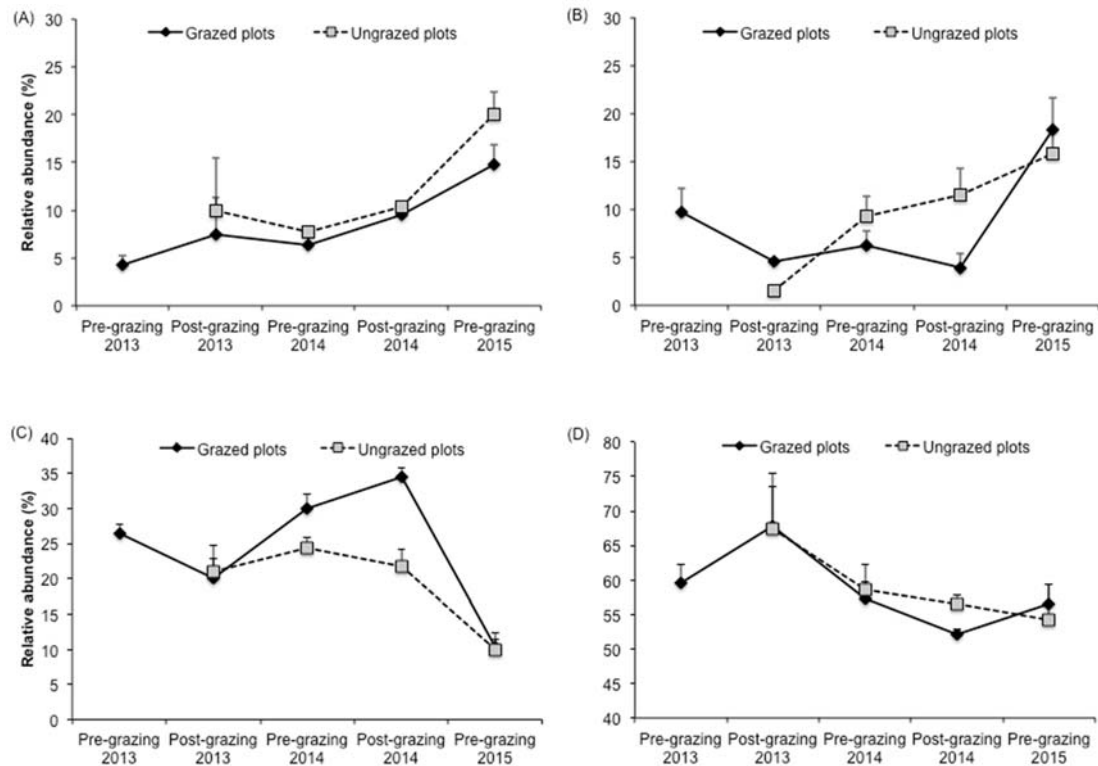
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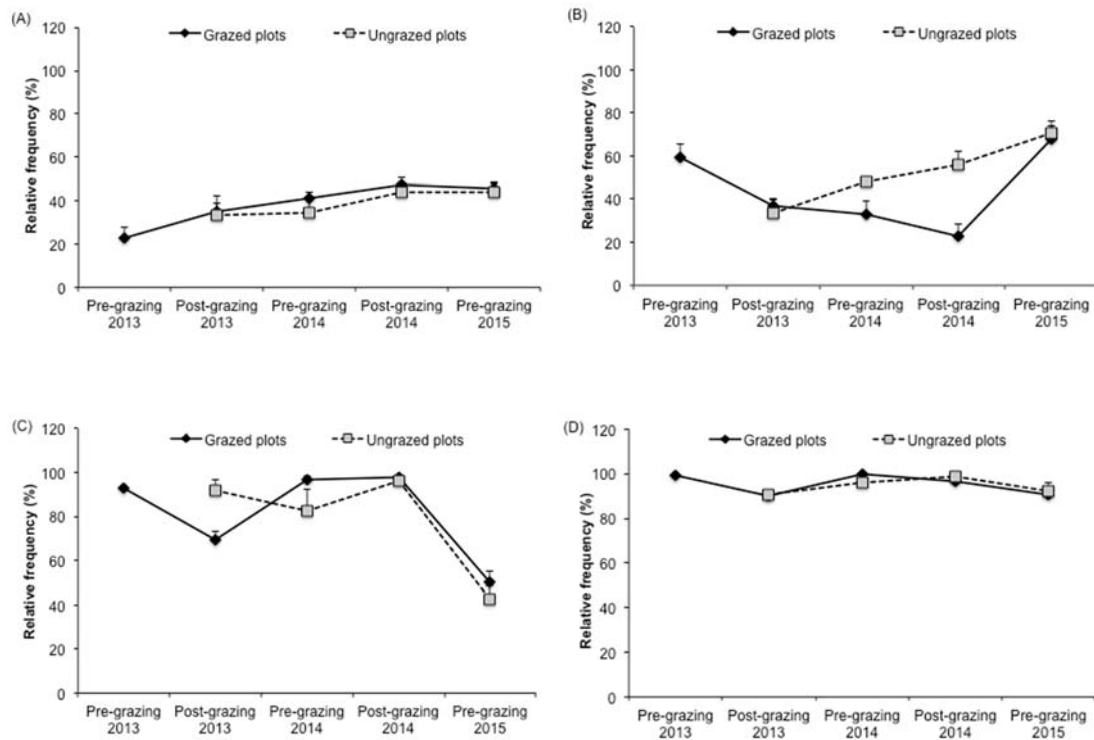
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## APPENDIX

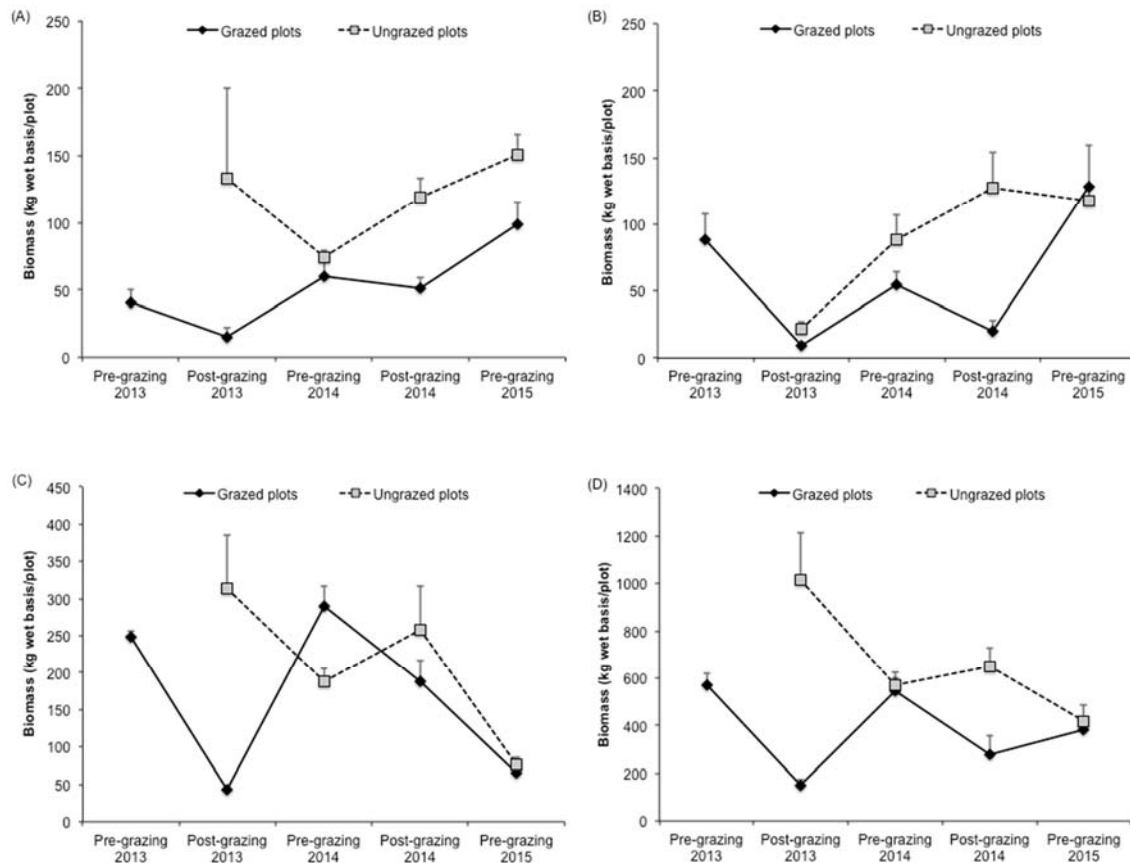
## CHANGES IN THE PLANT COMMUNITY DURING THE STUDY



**Figure A-1** Percentage of relative abundance of medusahead and plant functional groups in the plant community grazed by sheep during summer of 2013 (Chapter 2) and 2014 (Chapter 3): (A) medusahead; (B) other annual grasses; (C) bunch grasses, and (D) forbs. Relative abundance of plants was visually estimated along a zig-zag transect/plot (0.09 ha). Six grazed plots (Treatment) were sampled during 2013 and 10 grazed plots were sampled during 2014 and 2015. Three ungrazed plots (Control) were sampled during each year. The grazing period in 2013 was from June 2 to June 16 and the grazing period in 2014 was from June 10 to June 19. The estimation in 2015 was made from July 1 to July 3.



**Figure A-2** Percentage of relative frequency of medusahead and plant functional groups in the plant community grazed by sheep during summer of 2013 and 2014: (A) medusahead; (B) other annual grasses; (C) bunch grasses, and (D) forbs. Relative frequency of plants was visually estimated along a zig-zag transect/plot (0.09 ha). Six grazed plots (Treatment) were sampled during 2013 and 10 grazed plots were sampled during 2014 and 2015. Three ungrazed plots (Control) were sampled during each year. The grazing period in 2013 was from June 2 to June 16 and the grazing period in 2014 was from June 10 to June 19. The estimation in 2015 was made from July 1 to July 3.



**Figure A-3** Biomass (kg wet basis/plot [0.09 ha]) of medusahead and plant functional groups in the plant community grazed by sheep during summer of 2013 and 2014: (A) medusahead; (B) other annual grasses; (C) bunch grasses, and (D) forbs. Six grazed plots (Treatment) were sampled during 2013 and 10 grazed plots were sampled during 2014 and 2015. Three ungrazed plots (Control) were sampled during each year. The grazing period in 2013 was from June 2 to June 16 and the grazing period in 2014 was from June 10 to June 19. The estimation in 2015 was made from July 1 to July 3.

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 Utah State University, College of Natural Resources, Department of Wildland Resources.  
 Dissertation: Modulation of the nutritional context and early experience as new tools to increase the use of medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) by grazing sheep.  
 Advisor: Dr. Juan J. Villalba.
- 2008-2010 M.Sc. in Use, Management and Conservation of Natural Resources  
 Biological Research Center of the Northwest, Arid Zones Academy.  
 Dissertation: Determination of body, reproduction, and diet characteristics of feral pig (*Sus scrofa*) in the Sierra La Laguna Biosphere Reserve, Baja California Sur, Mexico.  
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**Experience**

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- Montes-Sánchez J.J.** and Buntinx-Dios, S. 2005. Determination of *n*-alkanes pattern in forages from semi-arid, temperate, humid tropical and dry tropical climate, during raining season. XII AMENA Congress, Puerto Vallarta, Jalisco, Mexico. Oral presentation.
- Montes-Sánchez, J.J.**, León-de la Luz, J.L., Buntinx-Dios, S., Huato-Soberanis, L., and Blázquez-Moreno, M.C. 2011. Selective foraging of feral pig *Sus scrofa* in the Sierra La Laguna Biosphere Reserve, Baja California Sur. XXVII Symposium of wildlife “Gral. MV Manuel Cabrera Valtierra”, Mexico City, Mexico. Oral presentation.
- Montes-Sánchez J.J.** and Villalba J.J. 2014. Effect of a high-energy supplement on medusahead-infested rangeland use by sheep. Society for Range Management Annual Meeting, Orlando, Florida, USA. Oral presentation.
- Montes-Sánchez J.J.** and Villalba J.J. 2016. Fermentation kinetic of medusahead and particle size reduction contribute to explain low intake and palatability. Society for Range Management Annual Meeting, Corpus Christi, Texas, USA. Oral presentation.



**Montes-Sánchez J.J.** and Villalba J.J. 2016. Influence of experience and availability of different forages on medusahead intake by sheep. Society for Range Management Annual Meeting, Corpus Christi, Texas, USA. Oral presentation.

## **Publications**

### *Journals*

- Revidatti, M.A., Delgado Bermejo, J.V., Gama, L.T., Landi Periat, V., Ginja, C., Alvarez, L.A., Vega-Pla, J.L. Martínez, A.M., and **BioPig Consortium**. 2014. Genetic characterization of local Criollo pig breeds from America using microsatellite markers. *Journal of Animal Science* 92, 4823-4832.
- Breceda, A., Arnaud-Franco, A., Álvarez-Cárdenas, S., Galina-Tessaro, P., and **Montes-Sánchez, J.** 2009. Evaluación de la población de cerdos asilvestrados (*Sus scrofa*) y su impacto en la Reserva de la Biosfera Sierra La Laguna, Baja California Sur, México. *Tropical Conservation Science* 2: 173-188.

### *Book chapter*

- Montes-Sánchez, J.J.**, León-de la Luz, J.L., Buntinx-Dios, S., Huato-Soberanis, L., and Blázquez-Moreno, M.C. 2012. Dieta, crecimiento y reproducción del cerdo asilvestrado *Sus scrofa* en la Reserva de la Biosfera Sierra La Laguna. In: Ortega-Rubio A., Lagunas-Vázquez, M., and Beltrán-Morales L.F. (Eds.), *Evaluación de la Reserva de la Biosfera Sierra La Laguna, Baja California Sur: avances y retos*. Centro de Investigaciones Biológicas del Noroeste, S.C. La Paz, pp. 183-204.

## **Awards**

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